The Journal of Laboratory and Clinical Medicine

VOL. 28 MAY, 1943 NO. 8

CLINICAL AND EXPERIMENTAL

BLOOD PRESSURE FLUCTUATIONS IN BRONCHIAL ASTHMA

I. CLINICAL OBSERVATIONS

HOWARD OSGOOD, M.D., BUFFALO, N. Y.

IN RECENT years there has been considerable discussion in medical literature on blood pressure in relation to bronchial asthma. This has been chiefly concerned with the question as to whether or not the mean systolic pressure shows a sustained rise or fall during an attack. It is not the purpose of the present paper to discuss this blood pressure change but rather to draw attention to certain fluctuations of the systolic pressure, which occur with each respiratory cycle during the asthmatic paroxysm, and to discuss their significance.

Minor fluctuations of the systolic blood pressure, synchronous with respiration, occur uniformly in normal individuals, the high point coming during expiration, the low point during inspiration in quiet breathing.

The respiratory fluctuations which occur in patients suffering from an attack of bronchial asthma are of greater amplitude. When the asthmatic paroxysm is severe, these fluctuations may be of sufficient magnitude to produce a noticeable waxing and waning of the radial pulse to palpation. A more common finding, however, is a lesser degree of fluctuation, not appreciable at the wrist, but only noted when the blood pressure is taken by a sphygmomanometer. The failure of these fluctuations to be detected ordinarily in the radial pulse may explain why they are seldom mentioned in medical texts dealing with allergic conditions or in general medical texts in connection with bronchial asthma. Physicians whose practice entails the observation of many asthmatic patients have undoubtedly noted them repeatedly.

From the Medical Services and Allergy Clinic of the Buffalo General Hospital, Buffalo, N. Y.

Received for publication, Sept. 8, 1942.

Sihle,[1] in 1903, stated that a majority of asthmatic patients during a paroxysm show a variable lowering of blood pressure, "so that in the same patient, in the course of five minutes, not infrequently values of 90, 70, 100, 85, 75 can be observed." He did not note that these were related to the respiratory cycle.

Tinel and Jacquelin[2] studied a number of asthmatic patients by means of a cuff over the humeral artery and kymograph tracings of the pulse. They noted the changes produced in the tracings by varying the pressure in the cuff. They found (1) that during the attack of asthma, coincident with inspiration, there was a considerable diminution of the amplitude of the pulse tracings, which was absolutely constant during the course of each paroxysm, and which ceased with its termination; (2) that there was a fall of the "maximum arterial tension" at the onset of dyspnea, amounting to 20 mm. or 30 mm. Hg, with a return to the former level with the end of the paroxysm; and (3) that an elevation of the "minimum arterial tension" occurred at the onset of the paroxysm, resulting in a dimunition of the oscillometric amplitude of the pulse. They concluded that these changes were due to some interference of the blood flow into the right heart and through the pulmonary circulation.

Rist, who discussed Tinel and Jacquelin's[2] paper, used the same method to determine the fluctuations of blood pressure which I have employed. He found that the systolic pressure (in various conditions) may vary as much as 30 mm. Hg between inspiration and expiration and maintained that the inspiratory suppression of the pulse noted by Tinel and Jacquelin was due to a lowering of the systolic pressure, and "was only an exaggeration of a normal phenomenon."

Rackemann[3] states that the respiratory fluctuations of the systolic blood pressure in asthma may be wide and are roughly parallel with the severity of the asthma. He also notes that there may be well-marked fluctuations in the presence of emphysema and many wheezes, when the severity of the asthma is not marked.

Feinberg[4] observes that during the asthmatic paroxysm, as a rule, the blood pressure rises and varies markedly with inspiration and expiration.

Detailed studies of the respiratory blood pressure fluctuations in bronchial asthma have not, so far as I am aware, appeared in the literature, and for this reason I present my findings.

METHOD

With practice, fluctuations of the systolic blood pressure in human beings, synchronous with the respiratory cycle, may be readily observed and measured by the auscultatory method, the stethoscope being placed over the antecubital space.

The usual 13 cm. cuff is applied to the upper arm and the sphygmomanometer placed close to the patient, so that it may be read and at the same time the patient's respirations can be seen "out of the corner of the eye." The cuff is inflated to a pressure above the highest systolic reading. The pressure is then slowly lowered, not more than 2 or 3 mm. per breath, the respiratory movements of the chest or abdomen being closely noted meanwhile. A point in the pressure will presently be reached, where a few beats will be heard at the elbow

at a certain moment in expiration. If the cuff pressure is held at this point, the auscultatory beats will disappear during the remainder of expiration and during the succeeding inspiration. There will be a trough of silence lasting until the corresponding moment in the next expiration, when the beats will reappear. The reading of the manometer at this point will indicate the highest level reached by the systolic pressure during the respiratory cycle.

As the cuff pressure is then further lowered slowly, more and more beats will be heard, first throughout expiration and then during inspiration. The manometer reading at the moment when beats are just heard throughout both inspiration and expiration marks the lowest point to which the systolic pressure falls during respiration. The difference between these two readings denotes in mm. Hg the amplitude of the respiratory fluctuations of the systolic pressure. The diastolic reading is taken in the usual way.

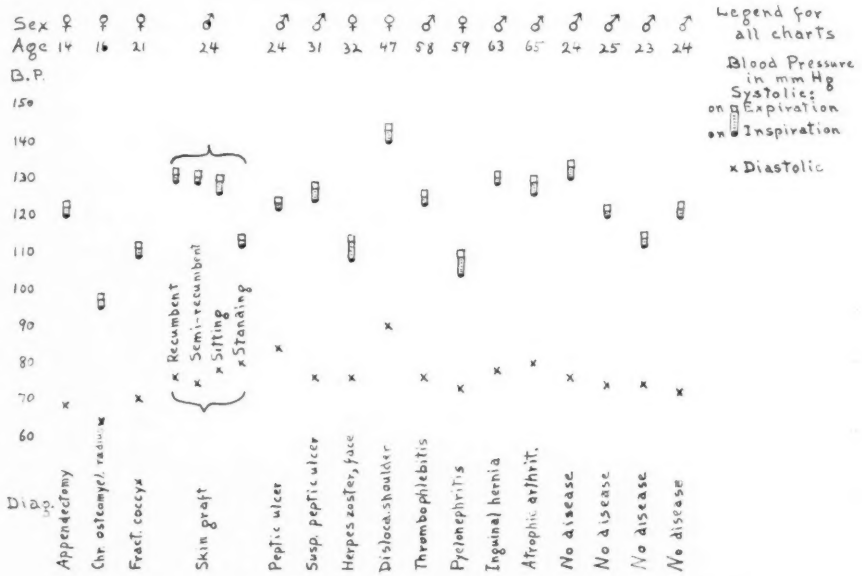


Fig. 1.—Normal respiratory fluctuations of the systolic blood pressure.

In my observations on asthmatic patients, and on normal individuals at rest, the high point of the systolic fluctuation has occurred uniformly during expiration and the low point during inspiration. It is possible that there may be circumstances where there is a reversal in phase of the high and low points, but I have not met with it. The diastolic pressure varies very slightly with respiration in asthma, but I have not included this variation in my studies. It is worth further attention.

In order to determine the normal limits of the respiratory fluctuation with this technique, determinations have been made on a number of subjects, both on healthy persons and on nonasthmatic hospital patients who were not acutely ill and were not suffering from cardiac, pulmonary, renal, or intrathoracic disease.

Fig. 1 shows the readings on sixteen such persons. The maximum fluctuation of the systolic pressure during respiration is 6 mm. Hg; the minimum is

2 mm. The average for the ten males is 3 mm.; for the six females it is 4 mm. Random observations on other similar individuals have shown an occasional fluctuation up to 12 mm. Hg in the absence of known disease.

OBSERVATIONS ON ASTHMATIC PATIENTS

Over the past three years determinations of the respiratory systolic fluctuation have been made on many asthmatic patients. The findings on some of these patients are here presented, with the high and low points of the systolic fluctuation indicated on the charts. The estimated severity of the asthmatic dyspnea at each reading is shown by the black columns at the bottom of the figures, with the dates beneath.

Fig. 2 shows findings on two patients on different days, the first reading in each being taken during an asthmatic paroxysm, the subsequent readings made when the paroxysm had subsided. In both, the fluctuation was wide when dyspnea was pronounced, diminishing when the dyspnea decreased.

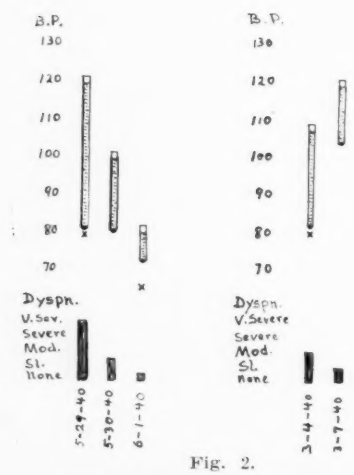


Fig. 2.

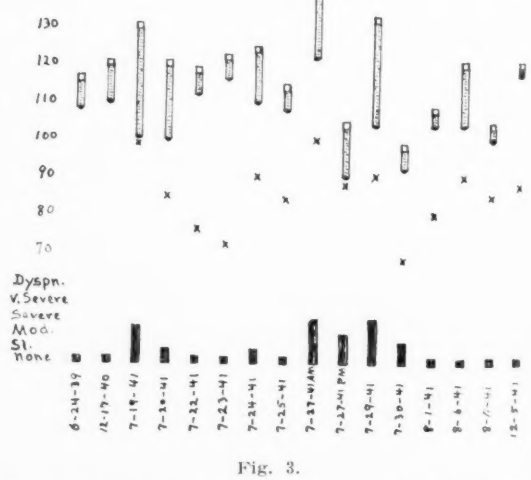


Fig. 3.

Fig. 2.—Respiratory systolic fluctuations at height of asthmatic paroxysm and after its subsidence. Patient B. B., female, 36 years of age, sensitive to inhalants and foods, and patient F. G., female, 38 years of age, sensitive to inhalants.

Fig. 3.—Respiratory systolic fluctuations in a chronic asthmatic patient, L. B., female, 48 years of age, with extrinsic and intrinsic factors as causative agents.

Fig. 3 presents readings taken at intervals over a period of two and one-half years on a chronic asthmatic patient whose asthma was due to intrinsic as well as inhalant factors. It will be noted that the respiratory systolic fluctuation followed closely the severity of the asthma, although on a few occasions it did not decrease to normal limits even though the patient was not apparently in distress. This corresponds to Rackemann's observation. One reading (third from the left) shows a fluctuation of 30 mm., the systolic pressure falling during inspiration almost to the diastolic level. This phenomenon has been noted in other patients when the asthma has been very severe.

Fig. 4 shows blood pressure readings taken over a period of a month (July 7 to August 7) on a patient 52 years of age who was very sensitive to grass and

weed pollens and to certain inhalants. During this month she passed through a very severe attack of asthma, being in an oxygen tent for five days. The respiratory systolic fluctuations parallel the severity of her asthma. Between July 20 and 29 there was a remission, at which time the fluctuations decreased to normal or near normal limits. A short recrudescence of the asthma then occurred with a corresponding increase in the systolic fluctuations, at one reading amounting to 30 mm. On August 7 she was asthma-free, and the fluctuation was 4 mm. Two readings in November, during a shorter attack of asthma, again disclosed an increase in the fluctuation. In this patient the mean systolic level tended to rise during a spell of asthma.

The determination of the respiratory systolic fluctuation has been useful in estimating the degree of bronchial obstruction present in a given patient at a given moment, as demonstrated in the following observations:

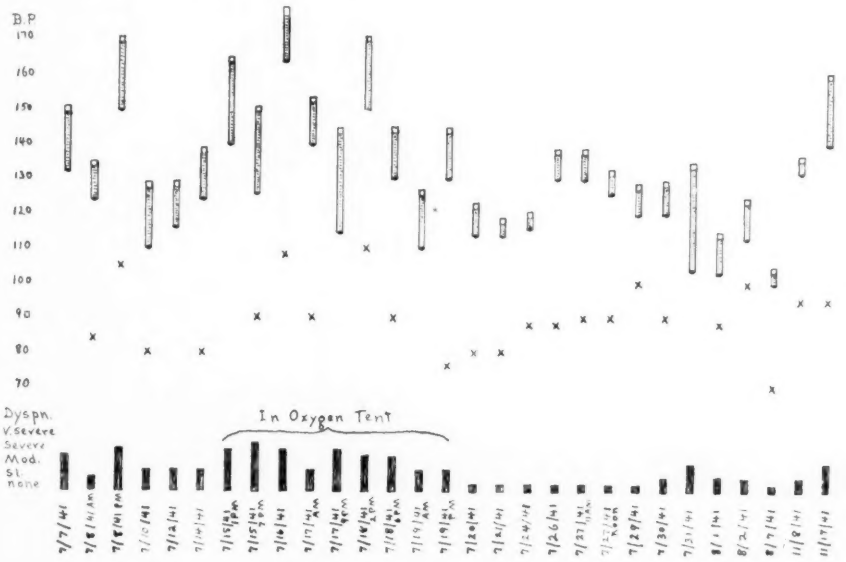


Fig. 4.—Respiratory systolic fluctuations throughout a prolonged attack of asthma. Patient G. H., female, 58 years of age, sensitive to pollens and inhalants.

An unmarried woman (Fig. 5), 53 years of age, was sensitive to orris, house dust, and grass pollen. She had had a number of moderately severe attacks of asthma and was highly apprehensive of its recurrence. A blood pressure reading during an asthma-free period yielded a respiratory systolic fluctuation of 5 mm. Three days later she complained of respiratory distress and a paroxysmal cough. When seen she was greatly excited and apprehensive, but to close observation she did not appear to be having much respiratory difficulty; an examination of her lungs showed only a few signs of bronchial spasm. The respiratory systolic fluctuation was 10 mm. With assurance and while preparations were being made to administer epinephrine in oil (but before it had been injected), her cough and distress subsided greatly. Ten minutes after the injection the respiratory systolic fluctuation was 7 mm. Here the distress was largely mental, and the blood pressure readings bore out the clinical impression that

there was little bronchial obstruction present. A stiff dose of sedative might have given as much relief as epinephrine, perhaps even more.

That relief of the bronchial asthma during an attack will bring about a reduction of the respiratory systolic fluctuation to within normal limits is demonstrated in Fig. 6. The patient was a married woman, 41 years of age who was primarily sensitive to orris root, with lesser sensitivity to house dust and flour mill dust. She had recurring attacks of severe asthma, but had developed no complicating conditions. Two blood pressure readings on December 2 and 4, when she was asthma-free, had shown respiratory systolic fluctuations of 3 and 4 mm., respectively. She was seen at 4 P.M. on January 13 in the midst of a

Fig. 5.—Respiratory systolic fluctuations in apparent asthmatic dyspnea but with little bronchial obstruction. Patient E. B., female, 53 years of age, sensitive to orris, dust and grass pollen.

severe attack; her lungs were full of wheezes. The respiratory systolic fluctuation was 30 mm. Injections of 0.25 c.c. epinephrine, 1 to 1,000, and 1 c.c. epinephrine in oil, 1 to 500, were given at 4:10 P.M. Some relief was noted at 4:20 P.M. and by 4:35 P.M. she was free of dyspnea, and the wheezes had disappeared from her lungs. Readings of the respiratory systolic fluctuation showed a progressive decrease in amplitude, until at 4:35 P.M. the fluctuation was 6 mm. A subsequent reading three months later, when she had no asthma, showed a fluctuation of 3 mm.

When adequate relief is not secured after palliative treatment, the continued dyspnea is reflected in a persistent wide respiratory systolic fluctuation.

In Fig. 7 are presented the findings in such an instance. The patient was a trained nurse, 43 years of age. She suffered from asthma at intervals throughout the year, with marked exacerbations during the grass season. Skin tests were negative except for a delayed positive to ragweed. She was seen on June 30 while in a severe asthmatic attack. The respiratory systolic fluctuation on two readings, taken ten minutes apart, were 26 and 45 mm., respectively. Injection of 0.6 c.c. epinephrine, 1 to 1,000, and 1 c.c. epinephrine in oil, 1 to 500, were then given. Five minutes after the injection she reported slight relief, and her respiratory systolic fluctuation had decreased to 16 mm. However, ten minutes later the relief had not been maintained and the fluctuation was 30 mm.

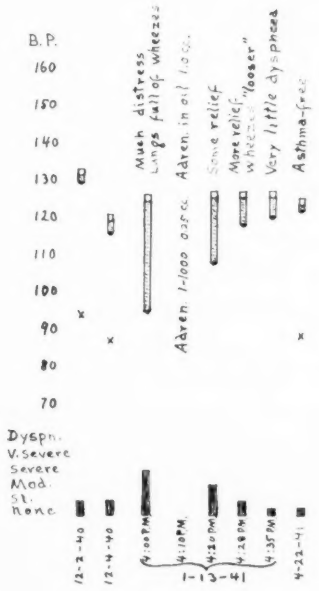


Fig. 6.

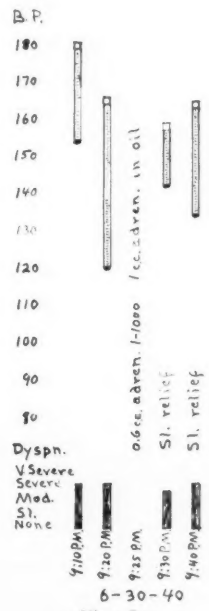


Fig. 7.

Fig. 6.—Respiratory systolic fluctuations before and after relief of an asthmatic paroxysm. Patient T. W., female, 41 years of age, sensitive to orris and other inhalants.

Fig. 7.—Respiratory systolic fluctuations when relief is not afforded by treatment. Patient M. M., female, 43 years of age, sensitive to pollens and inhalants.

DISCUSSION

The increased respiratory fluctuations of the systolic blood pressure reported above have been a constant finding in bronchial asthma.

A similar fluctuation (sharp fall in the systolic blood pressure) can be produced momentarily by attempting a deep inspiration with the nose and glottis closed (Mueller's experiment cited by Tinel and Jacquelin[2]). Certain experiments on cats, reported by me,[5] have shown fluctuations of the carotid blood pressure, similar to those in asthma, when obstruction to respiration was introduced at the trachea. Other experiments with increasing respiratory obstruction at the mouth in healthy young men, induced by progressively constricting a rubber mouthpiece, reported by me in the same paper,[5] also demonstrated an increasing fluctuation of the systolic blood pressure. In both cats and men, the amplitude of the fluctuations paralleled the degree of obstruction; and the

timing of the high and low points of the fluctuation in the respiratory cycle was the same as I have found it in asthmatic patients.

The mechanism by which this fluctuation is produced is probably complex. Reid,[6] in a recent article, has made some very interesting remarks on respiration as a factor in maintaining the circulation, which may throw light on the subject. The gist of his remarks is that during inspiration the intrathoracic pressure is lowered, favoring an inflow of blood from the large systemic veins into the chest, while at the same time the descent of the diaphragm raises the intra-abdominal pressure and so propels the blood through the vena cava into the large thoracic veins and to the right side of the heart. Also with the expansion of the lungs, the smaller pulmonary vessels dilate, so that a definite proportion of the whole blood volume is temporarily pooled in the vascular bed of the lungs. With the succeeding expiration, this pooled blood, plus the normal flow, is actively expelled from the lungs toward the left side of the heart by the elastic contraction of the lung tissue toward the hilum.

In bronchial asthma the intrathoracic and intra-abdominal pressure changes would be exaggerated. On *inspiration* it is possible that under the greater negative intrathoracic pressure[7] the vascular bed of the lungs dilates more than normal, pooling a greater amount of blood and withholding it from the left side of the heart. This would reduce the left ventricular output and so lower the systemic systolic pressure.

On *expiration,* which is considerably prolonged in bronchial asthma, there is an increased rise in the intrapleural pressure to even slightly above the atmospheric level.[7] This along with the elastic recoil of the lungs would induce an expulsion of the larger pool of blood in the lung-bed toward the left side of the heart, and a considerable increase of the left ventricular output would result, with a consequent rise in the systemic systolic blood pressure. Enhancing this greater supply of blood to the left side of the heart a moment later, but still during expiration, would be a greater supply of blood to the right side of the heart, as the increased intra-abdominal pressure, incident to the intense expiratory effort, propels more blood upward through the great veins into the chest. Since the intrathoracic pressure does not rise far above atmospheric pressure, whereas the intra-abdominal pressure starts at atmospheric level and is then increased by the vigorous action of the abdominal muscles in forced expiration, it is probable that this heightened pressure transmitted to the column of blood filling the vena cava would counteract any tendency of the positive pressure in the chest to collapse the veins entering the right side of the heart.

Whatever the true explanation, it is evident that the marked drop and subsequent rise of the systemic systolic blood pressure during the respiratory cycle in bronchial asthma is dependent upon some disturbance of the normal flow of blood through the lungs to the left auricle. This disturbance in turn is caused by the excessive fluctuations of the intrathoracic pressure induced by the obstruction to respiration in the smaller bronchi and bronchioles.

The parallelism between the presence and amplitude of the respiratory systolic blood pressure fluctuation on the one hand, and the severity of the asthma on the other, has been close, though not to a mathematical degree. I be-

lieve it may therefore be taken as an indirect and approximate measure of the degree of bronchial spasm or obstruction present and so may be a useful clinical sign. Conversely observations on the behavior of these fluctuations under various circumstances in bronchial asthma may offer a new method for studying the mechanism of the bronchial obstruction in this condition.

A paper is now in preparation covering observations on the effects of the administration of epinephrine and theophyllin ethylenediamine (aminophyllin) on these blood pressure fluctuations in asthmatic patients.

CONCLUSIONS

1. Wide fluctuations of the systolic blood pressure, synchronous with respiration, are a constant finding in bronchial asthma. The high point of the fluctuation occurs during expiration, the low point during inspiration.

2. The amplitude of these fluctuations parallels closely the severity of the asthma.

3. When the asthma has subsided, the fluctuations return to normal.

4. This respiratory systolic fluctuation is a useful clinical sign, indicating approximately the degree of respiratory obstruction present.

5. Observations on these fluctuations may be of value in studying the mechanism of bronchial asthma.

I wish to express my appreciation to Dr. Francis E. Ehret for his labor in going over innumerable records of asthmatic patients and abstracting the data on which this paper is based.

REFERENCES

1. Sihle, M.: Zur Pathologie und Therapie des Asthma, Wien. klin. Wchnschr. **16**: 85, 1903.
2. Tinel, J., and Jacquelin, A.: Les modifications circulatoires peripheriques au cours de L'acces d'asthme, Bull. et mém. Soc. méd. d. hôp. de Paris **47**: 440, 1931.
3. Rackemann, F. M.: Clinical Allergy, New York, 1931, The Macmillan Co., p. 431.
4. Feinberg, S. M.: Allergy in General Practice, Philadelphia, 1934, Lea & Febiger, p. 99.
5. Osgood, H., et al.: Blood Pressure Fluctuations in Respiratory Obstruction, Experimental Observations, J. Lab. & Clin. Med. **27**: 1536, 1942.
6. Reid, W. D.: Respiration as a Factor in the Circulation of the Blood, Ann. Int. Med. **17**: 206, 1942.
7. Prinzmetal, M.: Relation of Inspiratory Distention of the Lungs to Emphysema, J. Allergy **5**: 493, 1934.

U-WAVE PATTERNS IN THE ABNORMAL ELECTROCARDIOGRAM*

SYLVAN D. SOLARZ,† M.D., AND STEPHEN R. ELEK,‡ M.D., CHICAGO, ILL.

THERE has been a dearth of literature on the U wave of the electrocardiogram and little is known concerning its cause and significance. The literature has recently been reviewed by Papp[1] who defined the dimensions of this wave and stressed the frequency of inverted U waves in clinical heart disease. Nahum and Hoff[2] presented evidence from which they concluded that the U wave is related to the supernormal phase of the myocardium and that it marks the period when premature ventricular systoles occur most frequently. Recent studies, including those from this laboratory,[3, 4] showing that many abnormal electrocardiograms fall into definite patterns, suggested the desirability of determining the U-wave contours occurring in these various patterns.

The electrocardiograms were selected at random from the files of the heart station according to the electrocardiographic patterns. Most of the records had chest leads CF_2 and CF_4 as well as the limb leads. A few had only one chest lead (CF_2) and a few had no chest leads. Serial tracings, especially in the cases of myocardial infarcts, were available in many instances.

Three chief electrocardiographic patterns were analyzed: (a) ventricular preponderance (425 cases), diagnosed according to criteria established in this department;[3-5] (b) myocardial infarction (350 cases); and (c) intraventricular block (QRS duration over 0.12 sec.) (210 cases). A small series of cases of pericarditis (6 cases), of acute glomerulonephritis (3 cases) and of pulmonary embolism (6 cases) were also studied.

CRITERIA FOR THE NORMAL U WAVE

Optimal heart rates for the identification of the U wave are listed by Papp[1] as 60 to 110 beats per minute. Below this rate, the U wave tends to fuse with the T; above this rate it tends to be incorporated with the P wave. In the course of this study a number of records were found in which normal or abnormally tall, upright U waves followed so closely after the T wave as to simulate notched, diphasic, or bizarre T waves (Fig. 1-*A*, *B*). These can be distinguished by measuring the QRST interval of the other leads. In cases of tachycardia examples of amalgamation of U and P waves occurred (Figs. 2, 5-*A*). Here the distinction can be made by measuring the P-R interval of the other leads. Furthermore, in records of auricular fibrillation, with slow or moder-

*From the Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.
Aided by the A. D. Nast and Emily and Fanny Wedeles Funds for Cardiovascular Research.
Received for publication, Sept. 18, 1942.
†Sunset Camp Fellow. Now First Lieutenant, U. S. Army Air Force Medical Reserve.
‡Now First Lieutenant, U. S. Army Medical Reserve.

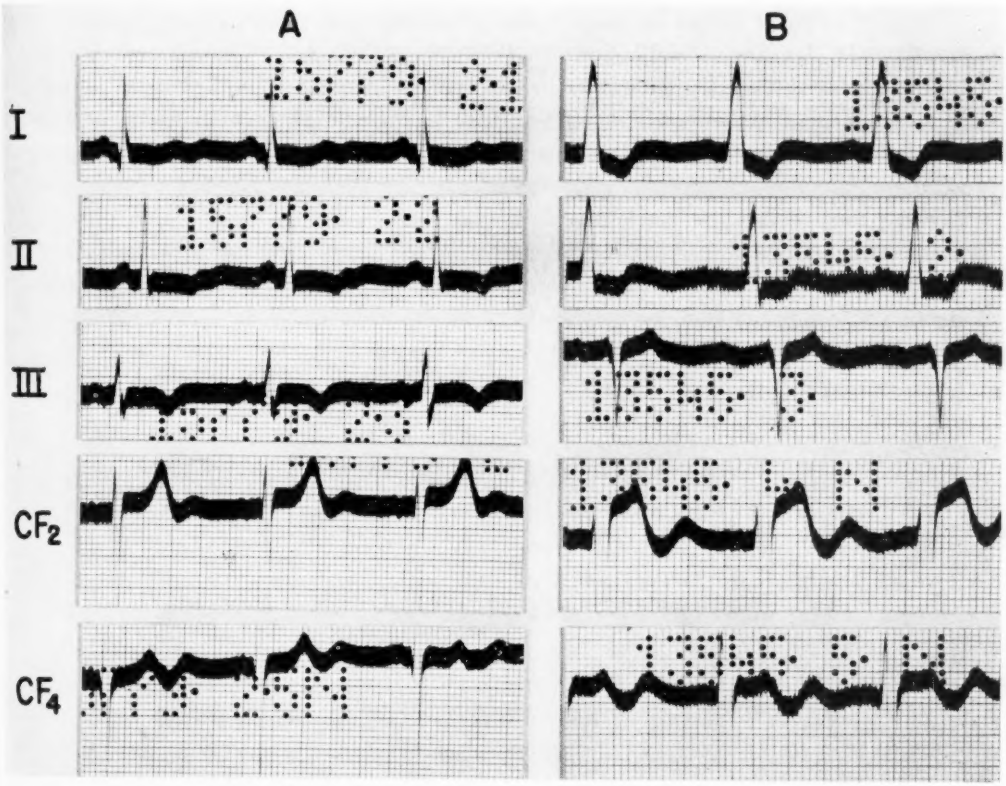


Fig. 1.—Simulated bizarre T waves caused by large U waves following closely after the T waves. *A*. In Leads I, II, CF_2 and CF_4 normal, upright U waves are seen giving the false impression of polyphasic T waves. *B*, Abnormally tall, upright U waves in the chest leads result in a configuration superficially resembling bizarre T waves.

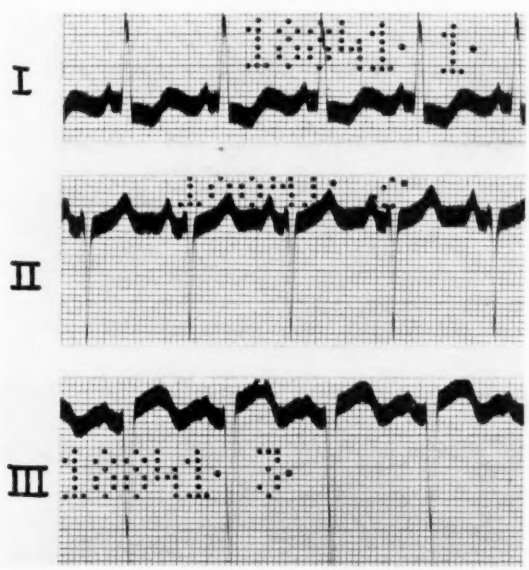


Fig. 2.—Amalgamation of the T and P waves in Lead III. This resembles a notched P wave. However, the P waves in all three leads are within normal limits and the P wave in Lead III can be located by comparing the P-R intervals in the several leads.

ately fast ventricular rates, large upright U waves may exist, especially in the chest leads, lending the illusion of P waves (Fig. 3). These observations indicate that the recognition of even the normal U wave is important in avoiding errors in description of the P and T waves and in measurement of the P-R interval. The error in diagnosis of mistaking a U for a P wave in cases of auricular fibrillation is, of course, evident. In addition, the presence of the U wave means that the isoelectric level can best be determined in the interval between U and P.

The criteria of Papp[1] for normality of the U wave were accepted in our study. The normal U wave is upright, varying in height from 0.25 to 2.0 mm.

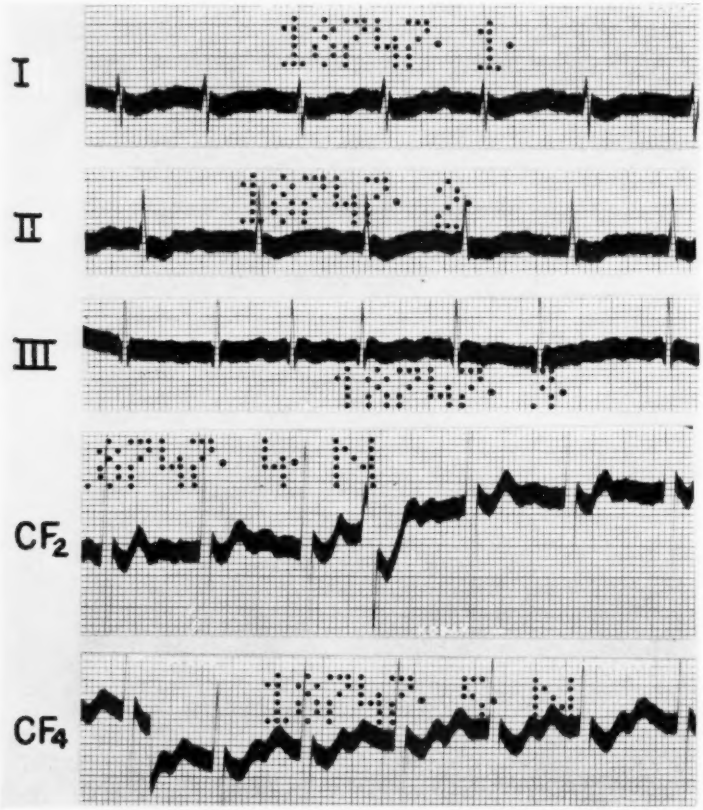


Fig. 3.—Auricular fibrillation of the coarse type. The U waves in leads CF_2 and CF_4 superficially give the impression of large P waves and might lead to an erroneous diagnosis.

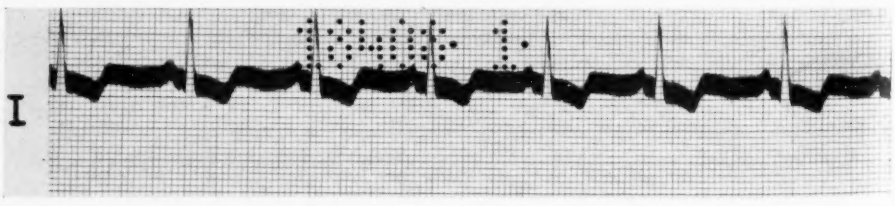


Fig. 4.—The downward bowed T-P segment in Lead I which is equivalent to an inverted U wave. Discussed in text.

and in duration from 0.18 to 0.24 sec. The U wave was considered abnormal when inverted, diphasic, or upright and taller than 2 mm. Another abnormality found by us especially in Lead I was the occurrence of a negative bowing of 1 mm. or more in the T-P segment of the electrocardiogram. In several records with this phenomenon the presence of a marked sinus arrhythmia soon made it evident that this bowing was due to the existence of an inverted U wave, for in the long T-P intervals the negative U wave was apparent; in the short T-P intervals only the downward bowing remained, the end of the U wave being invisible (Fig. 4). Hence this downward bowing of the T-P segment is regarded as an inverted U wave. A similar but normal upward bowing of the T-P segment may also be found.

THE U-WAVE PATTERNS IN HEART STRAIN

An analysis of 75 consecutive cases of right ventricular preponderance showed no abnormal U waves. Hence, the analysis of this pattern was not pursued further.

Examination of 350 cases of various types of left ventricular preponderance revealed 38 cases with abnormal U waves. Three cases were of the first type, 23 of the second type, 11 of the mixed type, and one of the concordant type.[4, 5]

The U-wave abnormality in the 3 cases of the first type of left ventricular preponderance consisted of U-wave inversion in CF_4 and on 2 occasions in Lead I. One instance of the inverted and one of the diphasic U were encountered in CF_2.

TABLE I

U-WAVE CONFIGURATION IN LEFT VENTRICULAR PREPONDERANCE OF THE SECOND TYPE IN THE 23 CASES SHOWING U-WAVE ABNORMALITIES

LEAD	U INVERTED	U UPRIGHT AND NORMAL	U UPRIGHT AND ABNORMAL	U DIPHASIC		U ISOELECTRIC
				1ST PHASE UP	1ST PHASE DOWN	
I	17	1	0	0	0	5
II	2	5	0	0	0	16
III	0	11	0	0	0	12
CF_2	3	13	1	2	0	4
CF_4	11	7	1	0	1	2*

*One record had no CF_4.

Table I summarizes the configuration of the U wave in the second type of left ventricular preponderance. U-wave inversion was most frequent in Lead I, occurred rarely in Lead II and was not encountered in Lead III. It occurred more frequently in CF_4 than in CF_2. In addition, in the chest leads abnormal upright and diphasic U waves were found. It would appear, therefore, that the idealized pattern of the U wave in this type of left ventricular preponderance consists of an inverted U_1, an upright or isoelectric U_2 and U_3, an upright U in CF_2 and an inverted U in CF_4 (Fig. 5-*A*). The lead distribution of the abnormal U waves in this pattern is shown in Table VII. It is evident from this table that U-wave abnormalities occurred most frequently in Lead I and next in frequency in Lead CF_4.

A similar trend is revealed in the mixed type of left ventricular preponderance (Tables II and VII). In the case of the concordant type of left heart strain the U was found to be inverted in Lead I.

THE U-WAVE PATTERNS IN INTRAVENTRICULAR BLOCK

An analysis of 75 consecutive cases of intraventricular block of the S type revealed no abnormal U waves. Hence, no further analysis was carried out in this group.

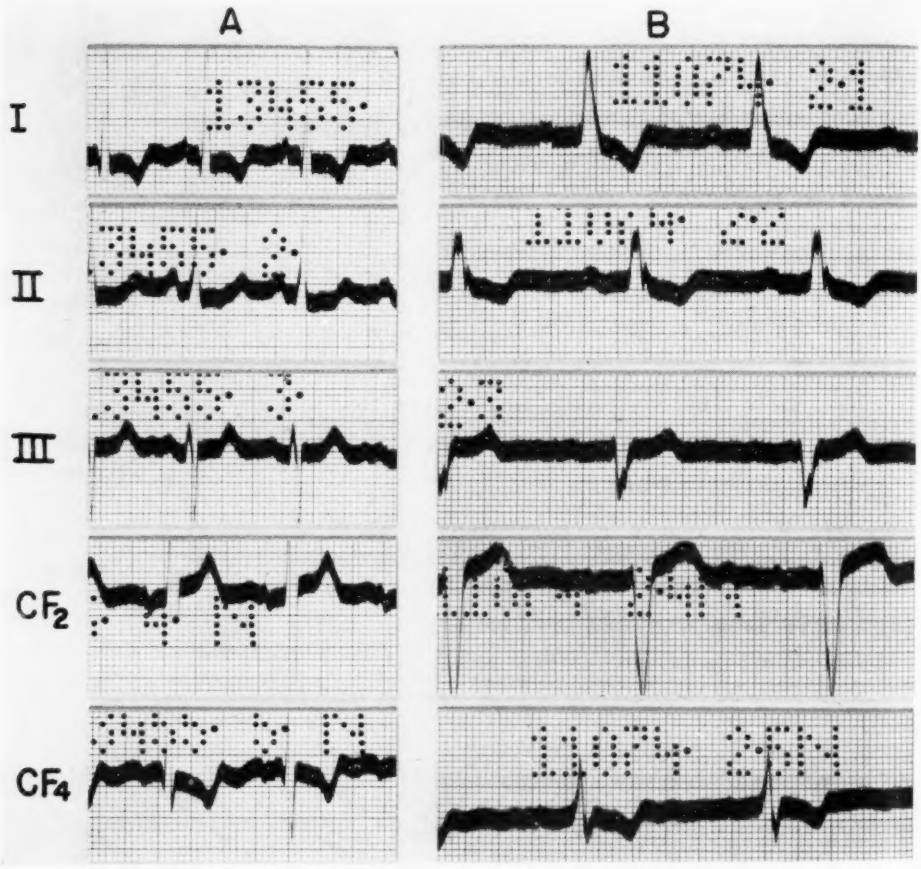


Fig. 5.—*A*, Second type of left ventricular preponderance. The U wave is inverted in Leads I and CF_4 and the T waves in these leads are also inverted. Note the U wave in Lead III is closely followed by the P wave and gives the false impression of a bizarre P wave (cf. Fig. 2). *B*, Intraventricular block of the common type. The U wave is inverted in Leads I and CF_4, and the T waves in these leads are inverted also.

Of the 135 cases of the common type of intraventricular block analyzed 17 had abnormal U waves; these findings are summarized in Table III. U-wave abnormalities occur most frequently in Leads I and CF_4 (Table VII). Thus a synthetic pattern in this type of intraventricular block would consist of inverted U waves in Leads I and CF_4 with normal upright or isoelectric U waves in Leads II and III and normal upright U waves in CF_2 (Fig. 5-*B*); this pattern is similar to that found in the second type of left ventricular preponderance.

TABLE II

U-WAVE CONFIGURATION IN LEFT VENTRICULAR PREPONDERANCE OF THE MIXED TYPE IN THE 11 CASES SHOWING U-WAVE ABNORMALITIES

LEAD	U INVERTED	U UPRIGHT AND NORMAL	U UPRIGHT AND ABNORMAL	U DIPHASIC		U ISO-ELECTRIC
				1ST PHASE UP	1ST PHASE DOWN	
I	8	0	0	0	0	3
II	1	2	0	0	0	8
III	0	4	0	0	1	6
CF_2	1	7	1	1	1	0
CF_4	5	5	0	0	1	0

TABLE III

U-WAVE CONFIGURATION IN COMMON TYPE OF INTRAVENTRICULAR BLOCK IN THE 17 CASES SHOWING U-WAVE ABNORMALITIES

LEAD	U INVERTED	U NORMAL AND UPRIGHT	U ISOELECTRIC	TOTAL CASES
I	13	0	4	17
II	1	5	11	17
III	0	7	10	17
CF_2	4	10	1	15*
CF_4	7	3	1	11†

*2 records had only 3 leads.
†6 records had no CF_4.

TABLE IV

U-WAVE CONFIGURATION IN ANTERIOR WALL INFARCTION PATTERN IN THE 18 CASES SHOWING U-WAVE ABNORMALITIES

LEAD	U INVERTED	U UPRIGHT AND NORMAL	U UPRIGHT AND ABNORMAL	U DIPHASIC		U ISO-ELECTRIC
				1ST PHASE UP	1ST PHASE DOWN	
I	6	0	0	0	0	12
II	1	4	0	0	0	13
III	0	10	0	0	0	8
CF_2	11	3	0	1	0	3
CF_4	16	0	0	1	0	1

TABLE V

U-WAVE CONFIGURATION IN POSTERIOR WALL INFARCTION PATTERN IN THE 11 CASES WITH U-WAVE ABNORMALITIES

LEAD	U INVERTED	U UPRIGHT AND NORMAL	U UPRIGHT AND ABNORMAL	U DIPHASIC		U ISO-ELECTRIC
				1ST PHASE UP	1ST PHASE DOWN	
I	3	2	0	0	0	6
II	1	2	0	0	0	8
III	2	2	0	0	0	7
CF_2	3	5	1	0	0	2
CF_4	9	1*	0	0	0	0

*One record had no CF_4.

THE U-WAVE PATTERNS IN MYOCARDIAL INFARCTION PATTERNS

Three hundred fifty cases with myocardial infarction patterns were analyzed, and in 39 abnormal U waves were seen. The latter included 18 of the anterior wall pattern, 11 of the posterior wall pattern, and 10 of the atypical patterns. Tables IV, V, and VI summarize the U-wave findings in these 39 cases.

In the anterior wall infarction series U-wave inversion was seen most frequently in CF_4 and next in frequency in CF_2 (Table VII). Thus, the idealized pattern of the U wave in anterior wall infarction consists of inversion of U in the chest leads, isoelectric or inverted U in Lead I, isoelectric U in Lead II, and isoelectric or upright U in Lead III (Fig. 6-*A*). Furthermore, analysis of 17 cases having serial curves showed that the U-wave abnormalities occurred only during the T stage and not the S-T stage.

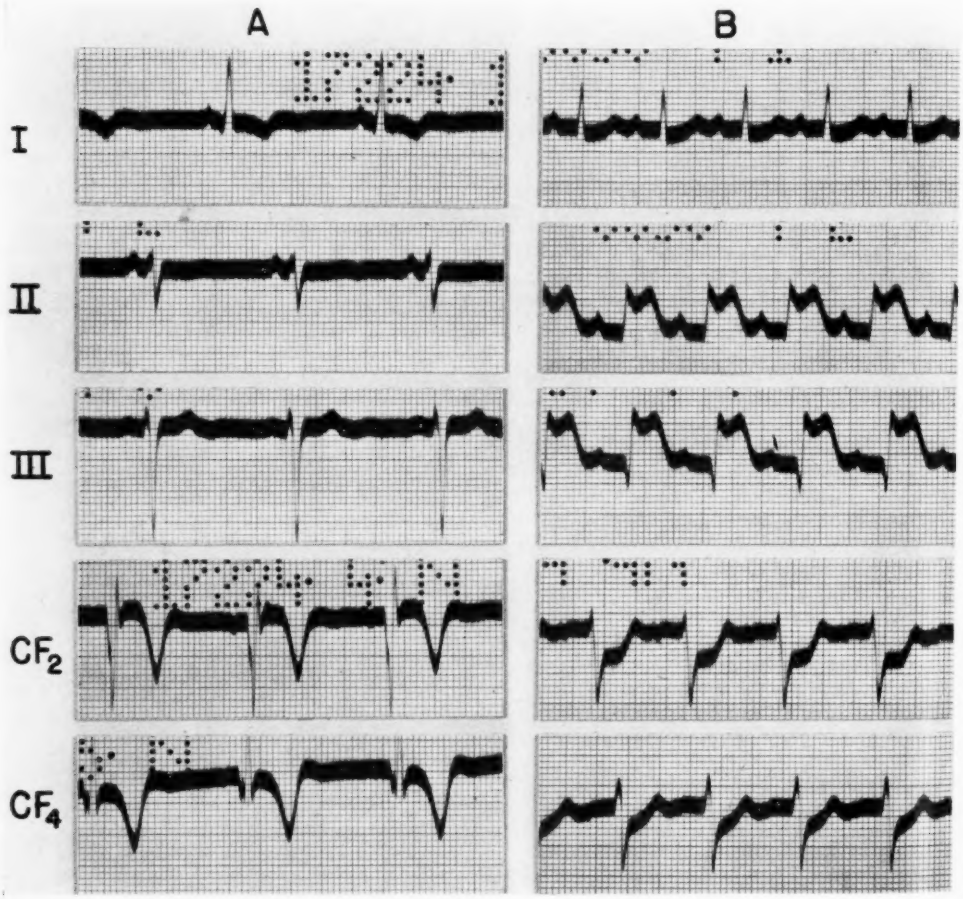


Fig. 6.—*A*. Anterior wall infarction in the healing (T) stage. The U wave in Leads I, CF_2 and CF_4 is inverted, and the U and T waves are concordant in direction in all leads. *B*. Very recent posterior wall infarction in the S-T stage (monophasic curve). The U waves in Leads CF_2 and CF_4 are inverted, and the S-T segments are markedly depressed. There is discordancy in direction between the U wave and T in these leads.

In the posterior wall infarction pattern Lead CF_4 again showed the greatest frequency of abnormal U waves (consisting of inversion) although scattered instances of abnormal U waves in the other leads were seen (Tables V and VII) (Fig. 6-*B*). Unlike the anterior wall infarcts, the U-wave abnormalities in posterior wall infarction were seen more commonly in the S-T stage.

TABLE VI

U-WAVE CONFIGURATION IN ATYPICAL MYOCARDIAL INFARCTION PATTERNS IN THE 10 CASES WITH U-WAVE ABNORMALITIES

LEAD	U INVERTED	U UPRIGHT AND NORMAL	U UPRIGHT AND ABNORMAL	U DIPHASIC		U ISO-ELECTRIC
				1ST PHASE UP	1ST PHASE DOWN	
I	2	1	0	1	0	6
II	1	2	0	0	0	7
III	4	5	0	0	0	1
CF_2	3	6	0	1	0	0
CF_4	8	0	0	2	0	0

TABLE VII

LEAD DISTRIBUTION OF ABNORMAL U WAVES IN THOSE INDIVIDUAL CASES SHOWING U-WAVE ABNORMALITIES

DIAGNOSIS	LEAD I	LEAD CF_4	LEADS I AND CF_4	LEAD CF_2	LEADS CF_2 AND CF_4	LEADS I, CF_2 AND CF_4	OTHER LEAD COMBINATIONS
Left Ventricular Preponderance, Mixed Type	4	0	2	0	3	1	1
Left Ventricular Preponderance, 2nd Type	9	4	4	0	2	1	3
Left Ventricular Preponderance, 1st Type	1	0	0	1	0	1	0
Left Ventricular Preponderance, Concordant Type	1	0	0	0	0	0	0
Intraventricular Block, Common Type	8	2	2	1	1	1	2
Anterior Wall Infarction Pattern	0	6	0	1	6	4	1
Posterior Wall Infarction Pattern	0	4	1	1	2	1	2
Atypical Infarction Patterns	0	4	1	0	4	0	1

Unlike the anterior and posterior wall patterns, the U-wave abnormalities in atypical infarction patterns occurred at all stages of the electrocardiographic evolution.

THE U-WAVE PATTERNS IN OTHER CONDITIONS

No abnormal U waves were found in the series of records of diffuse pericarditis, acute glomerulonephritis, or pulmonary embolism examined.

CORRELATION OF THE DIRECTION OF THE ABNORMAL U WAVES WITH THE DIRECTION OF THE T WAVE AND THE DEVIATION OF THE S-T SEGMENT

An impression was gathered in the course of this study that inverted U waves were usually associated with inverted T waves. In fact, Ashman and Hull[9] state that inverted U waves are commonly associated with inverted T waves. This was checked in all the records with regard to the concordancy of deviation of the U and T waves.

In left ventricular preponderance (all types) there was generally a concordancy in direction of the abnormal U waves and in the direction of T. This was most prominent in Leads I and CF_4 (Table VIII). This correlation was even more striking in the common type of intraventricular block since no instance of discordancy in direction of T and U occurred.

TABLE VIII

CORRELATION OF THE DIRECTION OF THE ABNORMAL U WAVE WITH THE DIRECTION OF THE T WAVE IN LEFT VENTRICULAR PREPONDERANCE AND IN THE COMMON TYPE OF INTRAVENTRICULAR BLOCK IN THE CASES WITH ABNORMAL U WAVES*

DIAGNOSIS	LEAD I		LEAD CF_2		LEAD CF_4	
	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT
Left Ventricular Preponderance, Mixed Type	8	0	3†	1	5	1
Left Ventricular Preponderance, Second Type	17	0	3†	3	11	3
Left Ventricular Preponderance, First Type	1	1	0	1	0	2
Left Ventricular Preponderance, Concordant Type	1	0	0	0	0	0
Intraventricular Block, Common Type	13	0	4	0	7	0

*A diphasic U wave was considered equivalent to an inverted one.
†2 U waves were inverted; one, upright and abnormal.

TABLE IX

CORRELATION OF DIRECTION OF ABNORMAL U WAVE WITH DIRECTION OF S-T DEVIATION AND WITH DIRECTION OF T WAVE IN CASES SHOWING ABNORMAL U WAVES*

DIAGNOSIS	S-T SEGMENT						T WAVE					
	LEAD I		LEAD CF_2		LEAD CF_4		LEAD I		LEAD CF_2		LEAD CF_4	
	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT	CONCORDANT	DISCORDANT
Anterior Wall Pattern	4	2	1	11	5	12	5	1	12	0	15	2
Posterior Wall Pattern	2	1	3	1	9	0	2	1	1	3	3	6
Atypical Patterns	1	2	0	4	5	5	2	1	3	1	7	3

*A diphasic U or T wave was considered equivalent to an inverted U or T.

In cases of anterior wall infarction pattern this concordancy was less striking but still fairly definite. However, in the posterior wall infarction pattern there was a definite discordancy in direction in T and U, especially in the chest leads. Accordingly, an analysis was made to ascertain a possible relationship between the direction of U and the deviation of the S-T segment.

In cases of anterior wall infarction there was a definite discordancy in the chest leads between the U and the deviation of the S-T segment (Table IX). A similar, although less definite, discordancy occurred in the series of atypical infarction patterns. On the other hand, in the posterior wall infarction patterns there was a definite concordancy in the direction of the U wave and the deviation of the S-T segment; this was most striking in Lead CF_4 (Table IX).

THE EVOLUTION OF THE U WAVE

An attempt was made to correlate the evolution of the abnormal U waves with that of the S-T-T segment in all cases having serial tracings.

In 15 of the 17 cases of left ventricular preponderance with serial tracings the abnormal U waves persisted. In the other 2 the abnormal U waves became normal even though the T waves remained inverted.

The U waves changed in appearance in 4 of the 8 cases of the common type of intraventricular block which had serial curves. In 2 of these the inverted U waves became isoelectric although the T waves remained inverted. The changes in the U wave in the other 2 cases were also independent of the T wave change.

In 6 of the 17 cases of anterior wall infarction pattern with serial tracings the abnormal U wave became normal. In 5 of these the change occurred in Lead CF_2, and, in all but one, the T wave in the lead remained inverted. It is significant that no abnormal U waves were seen when the S-T deviation was marked and the T wave small, even when the S-T deviation persisted for 10 months after the clinical attack.

In 5 of the 8 cases of posterior wall infarction pattern with serial records the abnormal U wave became normal. In contrast to the anterior wall infarction pattern U-wave abnormalities were noted in the posterior wall pattern during the period when the T was small and the S-T markedly deviated.

In 7 of the 9 cases of atypical infarction patterns with serial tracings the abnormal U waves changed to normal. These alterations in the U waves occurred without regard to the S-T or T changes.

It appears, therefore, that the initial appearance of the abnormal U wave is related to inversion of the T wave and to the depression of S-T in all the patterns studied except in myocardial infarction. In the latter, U inversion appears related to T inversion in the anterior pattern and to S-T depression in the posterior pattern. The U-wave evolution is sometimes independent of the S-T-T segment evolution.

Rapid appearance and disappearance of abnormal U waves were observed in 2 cases of transitory coronary insufficiency (with angina pectoris). A similar transitory occurrence of inverted U waves can be seen in 4 of the cases recently illustrated by Twiss and Sokolow.[6] In none of these 6 cases were records taken with sufficient frequency to determine whether or not the U-wave abnormality paralleled the abnormalities in the S-T-T segment. These cases do illustrate, however, how rapidly an abnormal U wave may appear and disappear.

DISCUSSION

Our results indicate that there is a tendency for the occurrence of a definite U-wave pattern in the abnormal electrocardiogram, although in most the U wave is normal in appearance in all the leads. When U-wave abnormalities occur in left heart strain and in intraventricular block of the common type the pattern consists, usually, of inverted U waves in Leads I and CF_4 with isoelectric or normal, upright U waves in the other leads. In anterior wall

infarction abnormal (inverted and diphasic) U waves occur most frequently in both Leads CF_2 and CF_4 with an occasional similar abnormality in Lead I, while in posterior wall infarction inverted U waves occur most commonly in CF_4 alone, although there are other scattered abnormalities. In all the patterns studied there was a general concordancy of the direction of the abnormal U wave with the direction of the T wave except in the early stages of posterior wall infarction pattern where there was a marked discordancy. In posterior wall infarction a definite concordancy existed with the deviation of the S-T segment as in other abnormal states expect in anterior wall infarction.

It must be re-emphasized that in these conditions many more instances of normal than of abnormal U waves were found.

The relationship of the abnormal U wave in posterior wall infarction to the deviation of the S-T segment rather than to the direction of the T wave is further supported by the rarity of abnormal U waves in Lead III in this pattern, in which lead, classically, the S-T segment is elevated and the T wave inverted. If abnormality of the U wave were related to the T-wave inversion, numerous instances of inverted U waves should be present in Lead III. On the other hand, in anterior wall infarction in which the S-T segment is depressed and the T wave is upright in Lead III abnormal U waves are not found, because, in this pattern, the U-wave abnormality appears to depend upon the direction of the T wave.

No abnormal U waves were found in the absence of other abnormalities in the electrocardiogram studied. Therefore, at the present, there is not much diagnostic value in the recognition of U-wave patterns; however, a description of the latter in abnormal records, such as has been attempted in this report, might aid in explaining the nature of the fundamental processes leading to the pattern of the U wave. Furthermore this knowledge will help to identify instances of distortion of the T and P waves and lead to a better definition of the isoelectric level.

SUMMARY

1. In 1000 cases with diagnostic electrocardiographic patterns, 94 cases of abnormal U waves were found. The U-wave patterns in these 94 cases showed the following chief characteristics:

(a) In left heart strain and in intraventricular block of the common type the U-wave abnormalities consist of inverted U waves in Leads II and CF_4.

(b) In anterior wall infarction abnormal (inverted or diphasic) U waves are found in Leads CF_2 and CF_4 with occasional similar abnormality in Lead I.

(c) In posterior wall infarction inverted U waves are found most commonly in Lead CF_4.

(d) No abnormal U waves were found in right heart strain or in intraventricular block of the indeterminate S type.

2. A hitherto undescribed U-wave abnormality, a negative bowing of the T-P segment in Lead I, is discussed.

3. Instances of distortion of the T and P waves by large upright U waves

and the confusion caused by the presence of prominent U waves in records of auricular fibrillation are pointed out.

4. Evidence is presented relating the presence of abnormal U waves to abnormalities of the S-T-T segment. In left heart strain and in the common type of intraventricular block, there is usually concordancy in the direction of the T wave, S-T deviation and U wave direction. In posterior wall infarction there is concordancy of the deviation of the S-T segment and the direction of the U wave but discordancy between T and U. In anterior wall infarction the reverse is true.

5. In serial records the abnormal U wave may undergo an evolution independent of the changes in the S-T-T segment.

We wish to express our grateful appreciation to Doctors L. N. Katz and R. Langendorf for their helpful criticisms.

REFERENCES

1. Papp, C.: U, the Sixth Wave of the Electrocardiogram, Brit. Heart J. 2: 9, 1940.
2. Nahum, L. H., and Hoff, H. E.: The Interpretation of the U Wave of the Electrocardiogram, Am. Heart J. **17**: 585, 1940.
3. Katz, L. N., Goldman, A. M., Langendorf, R., Kaplan, L. G., and Killian, S. T.: The Diagnostic Value of the Electrocardiogram Based on an Analysis of 149 Autopsied Cases, Am. Heart J. **24**: 627, 1942.
4. Kaplan, L. G., and Katz, L. N.: The Characteristic Electrocardiogram in Left Ventricular Strain With and Without Axis Deviation, Am. J. Med. Sci. **201**: 676, 1941.
5. Katz, L. N.: Electrocardiography, Lea & Febiger, Philadelphia, 1941.
6. Ashman, R., and Hull, E.: Essentials of Electrocardiography, ed. 2, Macmillan Company, p. 166, 1941.
7. Twiss, A., and Sokolow, M.: Angina Pectoris, Am. Heart J. 23: 498, 1942.

INACTIVATION OF VACCINIA VIRUS BY MILD ANTISEPTICS*†

WOLCOTT B. DUNHAM, M.D., AND WARD J. MACNEAL, M.D., NEW YORK CITY

LITTLE is known concerning the inactivating effect of antiseptics on the viruses. The present study was undertaken as one of a series to elucidate this subject. It seemed particularly desirable to investigate the action of antiseptics on the vaccinia virus, as this virus has been the most studied in other respects.

A number of reports have appeared on the effect of a wide variety of chemical and physical agents on the vaccinia virus. A partial summary of this literature is shown in outline form in Table I. It will be noted that this list contains few bacterial antiseptics and that in most of the investigations, the virus was exposed to the agent for an hour or more. In the tests to be described,

*From the Department of Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

†Aided by the Virus Research Fund of the Lambert Pharmacal Company, St. Louis, Mo.
Received for publication, Sept. 19, 1942.

TABLE I

RESULTS OF PREVIOUS INVESTIGATIONS ON THE INACTIVATION OF THE VACCINIA VIRUS

AGENT	CONCENTRATION	TIME	REMARKS	RESULTS[a]	REFERENCES
Phenol	5%	1 hr.	Diluted Calf Lymph	(+)	2
Phenol	1%	1 hr.	Diluted Calf Lymph	–	2
Mercuric Bichloride	1:5000	1 hr.	Diluted Calf Lymph	+	2
Mercuric Bichloride	1:10,000	1 hr.	Diluted Calf Lymph	–	2
Ethyl Alcohol	50%	1 hr.	Diluted Calf Lymph	+	2
Ethyl Alcohol	20%	24 hr.	Diluted Calf Lymph	–	2
Methyl Alcohol	50%	1 hr.	Diluted Calf Lymph	+	2
Methyl Alcohol	20%	1 hr.	Diluted Calf Lymph	(+)	2
Methyl Alcohol	10%	24 hr.	Diluted Calf Lymph	–	2
Potassium Permanganate	1:100,000	1 hr.	Diluted Calf Lymph	+	2
Hydrogen Peroxide	1:10	1 hr.	Diluted Calf Lymph	+	2
Hydrogen Peroxide	1:50	1 hr.	Diluted Calf Lymph	–	2
Sodium Salicylate	1:10	1 hr.	Diluted Calf Lymph	+	2
Sodium Salicylate	1:50	1 hr.	Diluted Calf Lymph	–	2
Potassium Salicylate	0.25 M	4 hr.	Tissue Extract	(+)	3
Potassium Salicylate	0.4 M	1 hr.	Tissue Extract	+	3
Ether	50%	24 hr.	Diluted Calf Lymph	(+)	2
Ether	20%	24 hr.	Diluted Calf Lymph	–	2
Acetone	50%	1 hr.	Diluted Calf Lymph	+	2
Acetone	10%	24 hr.	Diluted Calf Lymph	–	2
Formalin	0.25%	3 days	Washed Elementary Bodies	+	4
Chlorine	1:1,000,000	10 min.	Mandler Filtrate	–	5
Chlorine	1:500,000	10 min.	Mandler Filtrate	+	5
Hydrochloric Acid	N/1000	1 hr.	Diluted Calf Lymph	+	2
Sodium Hydroxide	N/500	1 hr.	Diluted Calf Lymph	+	2
Sodium Hydroxide	N/1000	24 hr.	Diluted Calf Lymph	–	2
Trypsin	0.5 mg. N per c.c.	3 wk.	Testicular Emulsion	–	6
Trypsin	0.5 mg. N per c.c.	1 wk.	Washed Elementary Bodies	(+)	6
Chymotrypsin	0.5 mg. N per c.c.	3 wk.	Washed Elementary Bodies	–	6
Saponin	0.2%	----	Chorio-allantoic Emulsion	(+)	7
Oil of Cloves	1:500	1 hr.	Diluted Calf Lymph	+	2
Drying	----	Years	Calf Lymph	–	8
Heat	55° C.	30 min.	Diluted Calf Lymph	+	2
Heat	50° C.	30 min.	Diluted Calf Lymph	–	2
Heat	100° C.	10 min.	Dry Virus	–	8
Cold	–8° C.	Years	Glycerinated Lymph	–	8
Pressure	400 Atmospheres	----	Glycerinated Lymph	–	8
Radium Radiation	----	----	----------	–	8
X-Ray	1.5 A	----	Testicular Emulsion	(+)	9
Light	Yellow, Green, Blue	----	Moist Virus	–	8
Light	Ultraviolet, Red	----	Moist Virus	+	8

[a] + Inactivation.
(+) Partial Inactivation.
– No Apparent Inactivation.

the vaccinia virus was exposed to the antiseptics for a period of only three minutes.

In a recent paper we[1] showed by means of chorio-allantoic inoculations that this virus is inactivated in 30 seconds by liquor antisepticus. The technic employed was rather time-consuming and it was not easy to make nearly simultaneous inoculations into a number of eggs. These difficulties were practically eliminated in the present study by injecting the mixtures of antiseptics and virus directly into the extra-embryonic fluids of the chick embryos. Two

advantages of this method are that a lot of 5 prepared eggs can be inoculated in half a minute and that the results tend to be remarkably uniform, apparently because the injurious effect of the needle puncture is slight. A disadvantage of this injection method is that localized pock lesions are seldom produced and hence one must, from time to time, test the virus by the chorio-allantoic method to be certain of its identity. Following inoculation into the extra-embryonic fluids, a generalized dissemination of the virus takes place and if the amount and activity of the inoculum are adequate, the embryo dies in two to four days. Death of the embryo is easily recognized by candling the eggs.

The virus suspension was prepared from incubated eggs which had died following injection of vaccinia virus CAEB, originally obtained from Doctor Joseph E. Smadel of the Rockefeller Institute. As a rule, the washed enveloping membrane of the yolk was used, but sometimes the tissue substance of the embryo itself and also the chorio-allantoic membrane were utilized. These tissues and membranes were ground with sand or glass granules and then mixed with Tyrode's solution, 1.5 c.c. for each yolk sac or chorio-allantois and 2 c.c. for each embryo body. The resulting suspension was centrifuged at 1,100 revolutions per minute for ten minutes. A pipette was then passed through the layer of yolk fat on top and the subjacent watery supernatant portion was withdrawn. This virus suspension was kept immersed in ice water until used. It was freshly prepared for each experiment and the virus strength was ascertained by titration of serial dilutions in each instance.

The eggs employed in the tests were incubated six to eight days, usually seven days. Before use they were candled and only those with vigorous embryos were employed. These were marked to indicate the location of the embryo and the border of the air sac. The blunt end of the shell was treated with iodine and then alcohol and by means of a 2 millimeter dental bur, a hole was drilled through the shell without piercing the shell membrane. Several eggs thus prepared were placed in a holder with the embryo uppermost until the inoculations had been completed.

The test solutions were kept at room temperature before use. The virus suspension was at a temperature of about plus 4° C. In all instances nine parts of the test solution and one part of the virus suspension were vigorously mixed and allowed to stand for three minutes. At the end of this period the mixture was injected into the eggs directly or sometimes after dilution with sterile distilled water. The decision in regard to the dilution depended on the results of the toxicity tests previously reported by us[10] in which eggs were injected with antiseptics alone. Some mixtures were injected directly, others diluted tenfold and others a hundred-fold.

Just before the end of the three-minute exposure period, the shell openings of the prepared eggs were wiped with alcohol. The test mixture was then drawn into a tuberculin syringe fitted with a 2 inch, 20 gauge needle. The needle was passed through the hole in the egg shell and directed slightly upwards so that its point was about 12 millimeters below the mark on the shell indicating the location of the embryo, and 0.05 c.c. of the material was in-

jected. Examination of eggs inoculated in a similar manner with insoluble material has shown that the inoculum enters the allantoic cavity, or, occasionally, the amniotic cavity. After the injection, the needle was withdrawn without sidewise motion and the other eggs of the series were inoculated in rapid succession. Leakage through the puncture did not occur. The holes in the shells were then sealed with liquid adhesive and the eggs were returned to the incubator.

For each experiment the concentration of virus in the vaccinia suspension was determined by making ten-fold dilutions in series with distilled water and injecting each dilution into eggs. Ordinarily this series of dilutions extended from 10^{-1} to 10^{-8}. The titer of the original suspension was taken to be the highest dilution which killed half the embryos inoculated. The number of minimal lethal doses (M.L.D.) in 0.05 c.c. of the original suspension was taken to be the reciprocal of the titer. Thus where half the eggs were killed by inoculation of 0.05 c.c. of the 10^{-5} dilution, the titer of the original suspension was 10^{-5} and there were 100,000 M.L.D. in 0.05 c.c. of this original suspension. In no instances were tests of antiseptics considered significant unless the eggs received at least 10 M.L.D. of the virus. Beyond this minimum, the actual con-

TABLE II

RESULTS OF INOCULATIONS OF EGGS WITH MIXTURES OF VACCINIA VIRUS AND ANTISEPTICS. REACTION PERIOD 3 MINUTES. FIVE EGGS IN EACH TEST

AGENT	CONCENTRATION	SUBSEQUENT DILUTION OF MIXTURE	MORTALITY IN 6 DAYS
Phenol	5%	100	4/15
Phenol	3%	100	6/14
Phenol	1%	10	15/15
Tincture of Iodine, U.S.P. XI	1%	100	1/15
Tincture of Iodine, U.S.P. XI	0.1%	10	15/15
Mercuric Bichloride	1:1000	100	4/14
Mercuric Bichloride	1:10,000	10	14/14
Mild Silver Protein	20%	100	14/15
Copper Sulfate	1%	100	10/14
Potassium Permanganate	1:1000	10	15/15
Propylene Glycol	70%	----	2/15
Propylene Glycol	60%	----	13/15
Propylene Glycol	50%	----	15/15
Ethyl Alcohol	25%	----	15/15
Boric Acid	4%	10	14/15
Boric Acid in 25% Alcohol	2.5%	----	15/15
Liquor Antisepticus, N.F. VI	100%	----	4/14
Liquor Antisepticus, N.F. VI	80%	----	14/15
Liquor Antisepticus, N.F. VI Revised[a]	100%	----	7/15
Listerine	100%	----	2/15
Listerine	80%	----	14/15
Metaphen	1:500	10	13/15
Tincture of Metaphen (1:200)	25%	10	7/15
Potassium Mercuric Iodide	1:5000	----	15/15
Merthiolate	1:1000	100	15/15
Tincture of Merthiolate (1:1000)	25%	10	15/15
Lysol	2%	10	3/15
Lysol	0.5%	----	10/15
Lysol	0.1%	----	15/15
Mercurochrome	2%	10	14/15
Amphyl	1%	----	3/15
Amphyl	0.5%	10	14/15

[a]Prepared according to the officially revised formula, effective July 1, 1940.[11]

centration of virus in the suspension was found to be without effect on the outcome of the tests.

After inoculation the eggs were candled daily for six days. When an embryo appeared to be dead, as evidenced by loss of vascular markings, cloudiness of the embryonic region and increased passive mobility of the embryo, the egg was opened and examined. In the rare instances where the embryo was found malformed or had obviously died from hemorrhage, these facts were recorded and the egg was excluded from consideration in the determination of experimental results. Survival of the embryo for six days was accepted as evidence that the material injected contained less than one lethal dose of the active virus.

Each test was repeated at least three times, usually on different days with a newly prepared virus suspension in each instance. The results are summarized in Table II.

DISCUSSION

A certain amount of irregularity should be expected in biological experiments of this kind and we have not regarded the death of one embryo in a lot of five as of great significance. Occasionally an egg died as the result of obviously recognizable trauma and could therefore be excluded from consideration. Doubtless, an occasional death from unrecognized trauma also occurred. Furthermore, the virus suspension was not of entirely uniform composition. Although only the supernatant suspension, after centrifuging, was used, nevertheless this might well contain clumps and scattered particles of tissue elements which would furnish protection for contained virus elements and which might be irregularly distributed in the final suspension. Because of the very brief period of exposure for inactivation, three minutes, some irregularity due to this factor might well be anticipated. However, these irregularities have been so insignificant that they did not obscure the results.

As shown in Table II, 3 per cent phenol was moderately effective, while a 1 per cent solution failed to inactivate the virus. Tincture of iodine was effective when diluted to 1 per cent strength. This solution contained iodine in a concentration of 0.7 mg. per c.c. When the official tincture was diluted to 0.1 per cent strength, it was without effect. Bichloride of mercury was effective in a concentration of 1:1000, though a 1:10,000 solution failed to protect the embryos. When mixtures prepared from mild silver protein and virus were injected into eggs, 14 out of 15 of the embryos died. Copper sulfate, 1 per cent, showed some virucidal action. Potassium permanganate, 1:1000, failed to inactivate the virus under the conditions of the tests. A high degree of protection was afforded by a 70 per cent solution of propylene glycol in water, while a 50 per cent solution was without demonstrable effect. The concentration of propylene glycol in these mixtures was 63 per cent and 45 per cent respectively by volume, or 66 per cent and 47 per cent by weight. Full strength liquor antisepticus was effective, though in tests conducted with an 80 per cent solution, all but 1 of the eggs died. It was shown that the action of liquor antisepticus was not due to the 25 per cent alcohol or to the boric acid alone, as these agents when tested separately did not inactivate the virus.

A combination of the two in the proportion in which they are present in liquor antisepticus also failed to protect the embryos. Listerine, full strength, inactivated the virus, while an 80 per cent solution gave results similar to the same dilution of liquor antisepticus, N. F. VI. Commercial tincture of metaphen 1:200, diluted to one-quarter strength, inactivated the virus to such an extent that more than half the eggs survived. On the other hand, after inoculation with mixtures prepared from virus suspension and an aqueous solution of metaphen, 1:500, 13 of the 15 eggs died. Potassium mercuric iodide, 1:5000; merthiolate, 1:1000; and the commercial tincture of merthiolate, 1:1000, diluted to one-quarter strength, were without demonstrable effect. One egg survived out of 15 injected with mixtures containing 2 per cent mercurochrome and virus. Lysol, 2 per cent, inactivated the vaccinia virus in the 3-minute period. A 0.5 per cent solution gave some protection to the embryos, while all the eggs died which received mixtures containing 0.1 per cent lysol. A 1 per cent solution of amphyl inactivated the virus, whereas a 0.5 per cent solution was without appreciable effect.

The virus suspensions employed in the tests contained a considerable amount of protein, from 1 to 2 Gm. per 100 c.c. The mixtures of antiseptic and virus, therefore, contained 0.1 to 0.2 Gm. per 100 c.c. The action of certain of the antiseptics would undoubtedly be modified by the presence of this nonvirus protein. In nature, the viruses are associated with variable amounts of nonvirus protein, a fact which makes it desirable to test the action of an agent in the presence of both large and small amounts of protein. The protein content of the virus suspensions employed in the tests here described is probably comparable to that of most of the suspensions used in the tests reported by others and summarized in Table I, as the virus for almost all of those studies was obtained by emulsifying infected tissue or by collecting the lymph from vaccinia vesicles.

In a companion investigation,[12] we determined the action of antiseptics on the influenza virus by a similar technic, employing as the virus suspension extra-embryonic fluid from eggs which had died following inoculation with this virus. The protein content of this suspension was about 4.6 mg. per 100 c.c. The difference in concentration of nonvirus protein in the two sets of tests must be borne in mind if any attempt is made to derive from these results a comparison of the resistance of the influenza and vaccinia viruses to antiseptics.

SUMMARY

A number of antiseptics were tested for their effect on the vaccinia virus during a brief period of exposure. This was accomplished by preparing mixtures of the antiseptics and virus, allowing them to remain in contact for 3 minutes, diluting the mixture to a point where it would not be toxic for chick embryos and then injecting the material into embryonated eggs. Survival of the embryos indicated inactivation of the virus.

The following agents were effective in inactivating the vaccine virus in 3 minutes: phenol, 3 per cent; tincture of iodine, 1 per cent; bichloride of

mercury, 1:1000; propylene glycol, 70 per cent; liquor antisepticus, N. F. VI, undiluted; listerine, undiluted; tincture of metaphen, 1:200, diluted to one-quarter strength; lysol, 2 per cent; and amphyl, 1 per cent.

REFERENCES

1. Dunham, Wolcott B., and MacNeal, Ward J.: Culture on the Chick Chorio-allantois as a Test of Inactivation of Vaccinia Virus, J. Bact. **44**: 413, 1942.
2. Gordon, M. H.: Studies of the Viruses of Vaccinia and Variola, Privy Council, Medical Research Council, Special Report Series, No. 98, London, 1925.
3. Cooke, Barbara, and Best, Rupert J.: Inactivating Effect of Salicylate on Suspensions of Some Animal Viruses. (Herpes, Infectious Myxoma, Shope Fibroma, Vaccinia and Poliomyelitis), Australian J. Exper. Biol. & M. Sc. **19**: 93, 1941.
4. Weil, A. J., and Gall, L. S.: Studies on the Immunization of Rabbits With Formalized Vaccine Virus, J. Immunol. **38**: 1, 1940.
5. MacDonald, Hugh: Chlorine Death Point of Cowpox Virus, Illinois M. J. **77**: 356, 1940.
6. Merrill, Malcolm H.: Effect of Purified Enzymes on Viruses and Gram-negative Bacteria, J. Exper. Med. **64**: 19, 1936.
7. Burnet, F. M., and Lush, D.: The Action of Surface-active Agents on Viruses, Australian J. Exper. Biol. & M. Sc. **18**: 141, 1940.
8. Paschen, E.: Pocken, Resistenzverhältnisse. Kolle, W., Kraus, R., and Uhlenhuth, P.: Handbuch der pathogenen Mikroorganismen, Ed. III, Band VIII: 870, Jena, Berlin, Wien, 1930, Gustav Fischer and Urban and Schwarzenberg.
9. Gowen, John W., and Lucas, Alfred M.: Reaction of Variola Vaccine Virus to Roentgen Rays, Science **90**: 621, 1939.
10. Dunham, Wolcott B.: Toxicity of Antiseptics for the Chick Embryo, Proc. Soc. Exper. Biol. & Med. **50**: 274, 1942.
11. Interim Revision Announcement, No. 2. National Formulary. Sixth Edition. J. Am. Pharm. **28**: 1061, 1939.
12. Dunham, Wolcott B., and MacNeal, Ward J.: Inactivation of Influenza Virus by Mild Antiseptics, J. Bact. **43**: 85, 1942.

THE COMPARATIVE EFFECTIVENESS OF ARSENICAL COMPOUNDS AND SULFONAMIDE DRUGS AGAINST BACTERIAL INFECTIONS*

EDWIN E. OSGOOD, M.D., PORTLAND, OREGON

WITH THE TECHNICAL ASSISTANCE OF JANE M. ARMENTROUT, M.A.; DOROTHY M. ELLIS, B.A.; INEZ E. BROWNLEE, B.A.; AND JULIA JOSKI, M.A.

IN PREVIOUS publications it has been shown, experimentally by the technique of marrow culture,[1] and clinically in staphylococcic bacteriemias[2] and cases of subacute bacterial endocarditis,[3] that neoarsphenamine is more effective than drugs of the sulfonamide group against the *Staphylococcus aureus* and most strains of the *Streptococcus viridans*. The present study was undertaken to determine in cultures of living human marrow cells the comparative effectiveness of neoarsphenamine, many other arsenicals, and the four sulfonamide drugs in most common use against the *Staphylococcus aureus*, the *Streptococcus viridans*, and many species of bacteria not previously reported on.

*From the Department of Medicine and the Division of Experimental Medicine, University of Oregon Medical School, Portland, Oregon.

Aided by grants from Mr. John C. Higgins, The Department of Clinical Research, Abbott Laboratories, and The General Research Council of the Oregon State System of Higher Education. The drugs used in the experiments were supplied by the Abbott Laboratories, the Lederle Laboratories, the Winthrop Chemical Company, and E. R. Squibb and Sons.

Received for publication, Sept. 21, 1942.

Clinically and in animal experimentation, control of such variables as the size of the initial inoculation, the number and strain of organisms present, the concentration of the drug in the blood and tissues during therapy, the day of disease on which therapy is started, the natural resistance of the host, and the presence or absence of antibodies is difficult, if not impossible, to attain. By the use of the method of human marrow culture[4] these variables are controlled and quantitative studies are possible. In addition, it is possible to study the toxicity of the drugs to living human cells.

METHOD

About 10 c.c. of marrow obtained by sternal puncture[5] or leukemic blood were introduced into 25 c.c. of citrated balanced salt solution in a 50 c.c. vaccine vial. After centrifugation the buffy coat was transferred to another vial containing balanced salt solution, and a total nucleated cell count was done and the culture diluted to a final volume of 50 to 100 c.c. containing 100,000,000 to 200,000,000 nucleated cells. The medium consisted of 65 per cent balanced salt solution similar in composition to cerebrospinal fluid and 35 per cent human cord serum or ascitic fluid. To this was added the desired inoculum of the bacteria to be studied and, after mixing, pour plates for colony counts were made on a medium suitable for the growth of the organism. These represent the initial colony counts at 0 time in the tables. These cultures were mixed again, and equal volumes were transferred to a series of six to fifteen 30 c.c. vaccine vials. All manipulations were made with syringe and needle through 70 per cent alcohol on vaccine caps. To each of these vials, except the control, the desired concentration of the drug to be studied was added, and a volume of saline equal to that in which the drugs were dissolved was added to the control vial. The vials were then transferred to an incubator at 37° C., and at intervals pour plates for colony counts and stained smears were made. This technique insures that the cultures were identical in every respect except for the single variable of the drug introduced.

RESULTS

A total of 211 such experiments on 118 different strains representing 11 different species of bacteria was performed. All of the 211 such experiments were analyzed in drawing the conclusions in this paper concerning the relative effectiveness of different drugs. Unless colony counts in the same experiment at the same time for different drugs or different concentrations of the same drug differed by a ratio of 10:1 they were not regarded as significantly different. In Tables I to V the results of 5 of the 211 experiments are tabulated.

Note from Table I that against this small inoculum of *Staphylococcus aureus* arsenic trioxide and the pentavalent arsenicals, acetarsone, aldarsone and carbarsone, are almost totally ineffective; that sulfathiazole in a concentration of 10 mg. per 100 c.c. is about equally effective with sulfadiazine at twice this concentration; and that all of the trivalent organic arsenicals used in this experiment are much more effective in the concentrations employed than are either sulfathiazole or sulfadiazine. In this particular experiment neoarsphenamine in a concentration of 0.5 mg. per 100 c.c. is about equal in effectiveness to 0.33

TABLE I

Experiment 614. *Staphylococcus aureus*, Lewis

HOURS	0	20	24	44
Control	84	1,000,000,000		
Neoarsphenamine, 0.5 mg. per 100 c.c.	84	360	960	5,100
Arsphenamine, 0.33 mg. per 100 c.c.	84	200	340	2,300
Mapharsen, 0.33 mg. per 100 c.c.	84	880	820	260,000
Mapharsen, 0.1 mg. per 100 c.c.	84	800	1,900	61,000,000
Trisodarsen, 0.5 mg. per 100 c.c.	84	2,700,000	12,000,000	400,000,000
Arsenic trioxide, 0.15 mg. per 100 c.c.	84	800,000,000		
Acetarsone, 0.4 mg. per 100 c.c.	84	1,000,000,000		
Aldarsone, 0.6 mg. per 100 c.c.	84	1,000,000,000		
Carbarsone, 0.4 mg. per 100 c.c.	84	800,000,000		
Sulfathiazole, 10 mg. per 100 c.c.	84	6,900,000	43,000,000	720,000,000
Sulfadiazine, 20 mg. per 100 c.c.	84	7,000,000		

TABLE II

Experiment 713. *Staphylococcus aureus*, Schildon

HOURS	0	20	46	68
Control	151	900,000,000	2,220,000,000	
Neoarsphenamine, 0.5 mg. per 100 c.c.	151	20	12	3
Arsphenamine, 0.3 mg. per 100 c.c.	151	600	400	60
Sulfarsphenamine, 0.33 mg. per 100 c.c.	151	360	100	35
Trisodarsen, 0.5 mg. per 100 c.c.	151	1,200	28,000	3,500
Mapharsen, 0.2 mg. per 100 c.c.	151	20	8	0
Mapharsen, 0.05 mg. per 100 c.c.	151	19,200	160,000,000	8,000,000,000
Clorarsen, 0.5 mg. per 100 c.c.	151	13	2	0
Clorarsen, 0.05 mg. per 100 c.c.	151	60,000	40,000,000	2,400,000,000
Sulfapyridine, 10 mg. per 100 c.c.	151	18,000,000	9,000,000,000	
Sulfathiazole, 10 mg. per 100 c.c.	151	80	13	5
Sulfadiazine, 10 mg. per 100 c.c.	151	100,000,000	5,000,000,000	

mg. per 100 c.c. of arsphenamine and only slightly superior in effectiveness to 0.33 mg. per 100 c.c. of mapharsen. It is far superior to 0.1 mg. per 100 c.c. of mapharsen and to 0.5 mg. per 100 c.c. of trisodarsen.

Note by comparison of Tables I and II that the strain of *Staphylococcus aureus* used in the experiment in Table II is far more susceptible to the action of all drugs than the strain employed in Table I. A larger inoculum is sterilized by concentrations of the drugs which failed to sterilize the strain used in Table I.

Note in Table II that in this experiment 0.5 mg. per 100 c.c. of neoarsphenamine, 0.2 mg. per 100 c.c. of mapharsen, 0.5 mg. per 100 c.c. of clorarsen, and 10 mg. per 100 c.c. of sulfathiazole are about equally effective; that 0.3 mg. per 100 c.c. of arsphenamine and 0.33 mg. per 100 c.c. of sulfarsphenamine are highly and equally effective; that 0.5 mg. per 100 c.c. of trisodarsen is definitely not as effective; and that mapharsen in a concentration one-tenth that of neoarsphenamine, which is about the ratio of their clinical doses, is not nearly as effective as neoarsphenamine but is more effective than an equal concentration of clorarsen. Also note how much less effective sulfapyridine and sulfadiazine are than is sulfathiazole in equal concentration.

Note in Table III that this strain of *Streptococcus viridans* is highly susceptible to the action of each of the trivalent organic arsenicals investigated but that clorarsen and mapharsen in one-tenth the concentration of neoarsphenamine are not nearly as effective.

TABLE III

Experiment 723. *Streptococcus viridans*, Winberg

HOURS	0	20	49	68
Control	33	185,000	2,800,000	
Neoarsphenamine, 0.5 mg. per 100 c.c.	33	5	2	0
Arsphenamine, 0.3 mg. per 100 c.c.	33	5	1	0
Sulfarsphenamine, 0.33 mg. per 100 c.c.	33	90	1	0
Clorarsen, 0.5 mg. per 100 c.c.	33	0	2	0
Clorarsen, 0.4 mg. per 100 c.c.	33	1	3	0
Clorarsen, 0.05 mg. per 100 c.c.	33	150	260	26
Mapharsen, 0.3 mg. per 100 cc..	33	1	1	0
Mapharsen, 0.2 mg. per 100 c.c.	33	4	0	0
Mapharsen, 0.05 mg. per 100 c.c.	33	1,700	12,500	700
Trisodarsen, 0.5 mg. per 100 c.c.	33	60	2	0

TABLE IV

Experiment 717. *Streptococcus hemolyticus*, Schuerman

HOURS	0	22	46
Control	4,600	19,000,000*	
Neoarsphenamine, 0.5 mg. per 100 c.c.	4,600	0	0
Arsphenamine, 0.3 mg. per 100 c.c.	4,600	0	0
Sulfarsphenamine, 0.33 mg. per 100 c.c.	4,600	0	0
Trisodarsen, 0.5 mg. per 100 c.c.	4,600	130	0
Mapharsen, 0.2 mg. per 100 c.c.	4,600	0	0
Mapharsen, 0.05 mg. per 100 c.c.	4,600	600,000	10,000
Clorarsen, 0.5 mg. per 100 c.c.	4,600	0	0
Clorarsen, 0.05 mg. per 100 c.c.	4,600	1,900,000	10,000
Sulfanilamide, 10 mg. per 100 c.c.	4,600	110,000,000	
Sulfapyridine, 10 mg. per 100 c.c.	4,600	70,000,000	
Sulfathiazole, 10 mg. per 100 c.c.	4,600	1,800,000	16,000,000
Sulfadiazine, 10 mg. per 100 c.c.	4,600	100,000,000	

*Acid at 18 hours.

TABLE V

Experiment 719. *Streptococcus hemolyticus*, Schuerman

HOURS	0	4	24
Control	54,000	10,000,000	40,000,000
Neoarsphenamine, 0.5 mg. per 100 c.c.	54,000	7,000	0
Arsphenamine, 0.3 mg. per 100 c.c.	54,000	30,000	10
Sulfarsphenamine, 0.33 mg. per 100 c.c.	54,000	40,000	0
Mapharsen, 0.3 mg. per 100 c.c.	54,000	5,600	0
Mapharsen, 0.2 mg. per 100 c.c.	54,000	9,000	0
Mapharsen, 0.05 mg. per 100 c.c.	54,000	100,000	210,000
Clorarsen, 0.5 mg. per 100 c.c.	54,000	4,200	0
Clorarsen, 0.4 mg. per 100 c.c.	54,000	5,000	0
Clorarsen, 0.05 mg. per 100 c.c.	54,000	200,000	90,000

Note in Table IV that the *Streptococcus hemolyticus* (beta hemolytic streptococcus, Lancefield group A) is far more susceptible to the action of the trivalent organic arsenicals than is the staphylococcus, since a larger inoculum is sterilized by the same concentrations of the drugs which failed to sterilize small inoculums of the Staphylococcus aureus. It is again evident in this experiment that trisodarsen in equal concentration is not as effective as neoarsphenamine; that mapharsen in one-tenth the concentration is not nearly as effective as neoarsphenamine; that the trivalent organic arsenicals are all far superior to the sulfonamide drugs; and that sulfathiazole is much the most effective of the sulfonamide drugs.

Note from a comparison of Tables IV and V that it is necessary to increase the initial inoculum to demonstrate the differences in effectiveness of the most effective drugs and to decrease the initial inoculum to demonstrate the differences in effectiveness of the least effective drugs.

Note in Table V the great susceptibility of this strain of *Streptococcus hemolyticus* to the trivalent organic arsenicals and that 0.5 mg. per 100 c.c. of neoarsphenamine is about equal in effectiveness to 0.25 mg. of mapharsen and 0.4 mg. per 100 c.c. of clorarsen; that 0.3 mg. per 100 c.c. of arsphenamine and 0.33 mg. per 100 c.c. of sulfarsphenamine are almost as effective but that one-tenth the concentration of mapharsen and clorarsen are not nearly as effective.

COMMENT

Analyses of the 211 experiments on the 118 strains representing 11 different species of bacteria were made. The pentavalent organic arsenicals studied and arsenic trioxide were almost totally ineffective when compared with effective concentrations of neoarsphenamine, either in the ratios of their clinical doses or in concentrations which would give an equal arsenic content. The pentavalent arsenicals studied included acetarsone, carbarsone, aldarsone, para-sulfonamindophenyl arsonic acid, tryparsamide, sodium cacodylate, and solarson. Only solarson seemed to be of any value at all, and this was far less effective than neoarsphenamine.

All of the trivalent organic arsenicals tested were highly effective against certain species of bacteria in concentrations of equal arsenic concentration to the effective range for neoarsphenamine previously determined to be 75 to 200 gammas (micrograms) per 100 c.c.,[6] corresponding to 0.375 to 0.1 mg. per 100 c.c. of neoarsphenamine. The trivalent organic arsenicals tested were neoarsphenamine, arsphenamine, sulfarsphenamine, trisodarsen, mapharsen, clorarsen, bismarsen, and silver arsphenamine. The latter two offered no advantages for clinical use, since they were relatively ineffective in comparison to the other arsenicals; so they were not studied very extensively.

The different species of bacteria and in some instances even different strains of the same species differed markedly in their susceptibility to the action of the drugs. When an organism was susceptible to the action of one of these arsenicals the same strain was always highly susceptible to the action of the other trivalent organic arsenicals when compared in doses having an equal arsenic content. When the organism was resistant to the action of one arsenical it was resistant to the action of the others as well, although this was not extensively investigated. While there were individual strain differences in susceptibility with each of the organisms tested, only with the *Streptococcus viridans* was there a sufficient difference to justify grouping these strains into separate categories. As pointed out in previous publications,[6] some strains of *Streptococcus viridans* respond to neoarsphenamine and not at all to the sulfonamide drugs, and these strains have arbitrarily been called "Group A." Other strains are susceptible both to the action of neoarsphenamine and to the sulfonamide drugs, and these have been arbitrarily called "Group B." Other strains have been susceptible to the action of the sulfonamide drugs and not to neoarsphenamine, and these have arbitrarily been called "Group C."

In Table VI, the response of the bacteria tested to the trivalent organic arsenicals is summarized. Note that the *Staphylococcus aureus*, *Streptococcus viridans* (Groups A and B), *Streptococcus hemolyticus* (betahemolytic streptococcus, Lancefield group A), *Corynebacterium diphtheriae*, *Neisseria gonorrhoeae*, and *Hemophilus influenzae* are highly susceptible to the action of these drugs; whereas, the *Diplococcus pneumoniae*, *Escherichia coli*, *Eberthella typhosa*, *Salmonella schottmuelleri*, *Streptococcus viridans* (Group C), and *Streptococcus anhemolyticus* (gamma streptococcus) were almost totally unaffected by the same concentrations of these trivalent organic arsenicals. Most strains of the first six species of bacteria in Table VI were far more susceptible to the action of the trivalent arsenicals in the concentrations employed than they were to the usual concentrations of the sulfonamide drugs obtainable in the blood stream. *Neisseria gonorrhoeae*[7] and *Hemophilus influenzae*, however, were somewhat less susceptible to the action of the trivalent organic arsenicals than to sulfathiazole, sulfadiazine, and sulfapyridine, although they were more susceptible to the action of the trivalent arsenicals than to the action of sulfanilamide.

TABLE VI

SUMMARY OF THE RESPONSE OF BACTERIA TO TRIVALENT ORGANIC ARSENICALS

ARSENICALS EFFECTIVE	ARSENICALS INEFFECTIVE
Staphylococcus aureus	*Diplococcus pneumoniae*
Streptococcus viridans	*Escherichia coli*
Group A	*Eberthella typhosa*
Group B	*Salmonella schottmuelleri*
Streptococcus hemolyticus	*Streptococcus viridans*
Corynebacterium diphtheriae	Group C
Neisseria gonorrhoea	*Streptococcus anhemolyticus*
Hemophilus influenzae	

Against each of the organisms listed in Table VI, sulfathiazole was the most effective of the sulfonamide drugs and sulfanilamide was the least effective. All of these organisms, except the Group A *Streptococcus viridans*, showed some response to 10 mg. per 100 c.c. of sulfathiazole if small enough initial inoculums were used. Sulfapyridine was superior to sulfadiazine in equal concentration against most strains of all of the species in Table VI, except *Neisseria gonorrhoeae* and *Salmonella schottmuelleri* where sulfadiazine was as a rule superior to sulfapyridine.

While there were rather marked individual strain differences among organisms of the same species or group there was a very great variation in susceptibility of the different species of organisms to the trivalent organic arsenicals and to the sulfonamide drugs, as indicated by the maximum initial inoculums which could be sterilized.

Note from Table VII that the *Streptococcus hemolyticus* is the most susceptible organism to the action of the trivalent organic arsenicals and that the *Neisseria gonorrhoeae* is the most susceptible organism to the action of the sulfonamide drugs. For each group of drugs the organisms can be divided into five or six groups of markedly different susceptibilities.

Since six of the trivalent organic arsenicals tested were about equally effective in terms of equal arsenic content, it seemed desirable to calculate a thera-

TABLE VII

COMPARATIVE SUSCEPTIBILITY OF ORGANISMS TO NEOARSPHENAMINE AND SULFATHIAZOLE

NEOARSPHENAMINE, 0.5 MG. PER 100 C.C.		SULFATHIAZOLE, 5-10 MG. PER 100 C.C.	
ORGANISMS	MAXIMUM INOCULUM STERILIZED	ORGANISMS	MAXIMUM INOCULUM STERILIZED
Str. hemolyticus	800,000	*N. gonorrhoeae*	1,000,000
N. gonorrhoeae	20,000	*Str. hemolyticus*	6,000
Str. viridans (Groups A and B)	1,500-3,000	*Str. viridans* (Groups B and C)	1,500-3,000
H. influenzae	1,500-3,000	*Dip. pneumoniae*	1,500-3,000
C. diphtheriae	1,500-3,000	*H. influenzae*	1,500-3,000
Staph. aureus	750	*Staph. aureus*	100-300
Sal. schottmuelleri	Relatively ineffective	*C. diphtheriae*	100-300
E. typhosa	Relatively ineffective	*Sal. schottmuelleri*	100-300
E. coli	Relatively ineffective	*E. typhosa*	100-300
Str. anhemolyticus	Relatively ineffective	*E. coli*	100-300
Dip. pneumoniae	Relatively ineffective	*Str. anhemolyticus*	100
Str. viridans (Group C)	Relatively ineffective	*Str. viridans* (Group A)	Ineffective

TABLE VIII

COMPARATIVE EFFECTIVENESS OF THE TRIVALENT ORGANIC ARSENICALS

DRUG	PERCENTAGE OF ARSENIC	EQUALLY EFFECTIVE CONCENTRATION		MAXIMUM THERAPEUTIC DOSE		THERAPEUTIC INDEX	
		MG. PER 100 C.C.	GAMMAS, AS. PER 100 C.C.	GM. PER 60 KG.*	MG. PER 100 GM.		NEO = 100
Neoarsphenamine	20.0	0.5	100	0.9	1.5	3.0	100.0
Sulfarsphenamine	19.0	0.5	95	0.6	1.0	2.0	66.7
Arsphenamine	31.0	0.35	110	0.4	0.67	1.9	63.3
Trisodarsen	19.0	0.6	114	0.6	1.0	1.67	55.7
Mapharsen	29.0	0.25	75	0.06	0.1	0.4	13.3
Clorarsen	25.5	0.4	102	0.067	0.11	0.275	9.0

*From Moore, J. E., et al.: The Modern Treatment of Syphilis, p. 76. Charles C Thomas, Springfield, 1941.

†Mg. per 100 Gm. (dose) ÷ Mg. per 100 c.c. (concentration).

peutic index to determine which of them should be most promising for clinical use. It has been previously determined[6] that the effective range of neoarsphenamine in clinical use is 0.375 to 1.00 mg. per 100 c.c. of blood corresponding to 75 to 200 gammas (micrograms) of arsenic per 100 c.c. Therefore, neoarsphenamine in a concentration of 0.5 mg. per 100 c.c. corresponding to 100 gammas of arsenic per 100 c.c. was used as a reference standard, and all experiments in which this concentration of neoarsphenamine was compared with one or more of the other five arsenicals were analyzed. From these data, Table VIII was prepared. The first vertical column in Table VIII lists the commonly used names of the six trivalent organic arsenicals which offer most promise in the treatment of bacterial infections. The second column gives the percentage of arsenic in the drug as it is supplied in ampoules, which is not always the percentage as it is calculated from the structural formula. The third column gives the concentration in milligrams per 100 c.c. of these drugs as commercially supplied which, from analysis of all of the experiments in which the drugs were compared, appeared to be equivalent to 0.5 mg. per 100 c.c. of neoarsphenamine. The fourth column gives the gammas (micrograms) of arsenic per 100 c.c. which correspond to the amount of drug listed in the third column. From examination of this

column it is apparent that the drugs are approximately equally effective in concentrations of equal arsenic content, with the exception of mapharsen which is slightly more effective than the others in terms of equal arsenic content. The fifth column is the maximum therapeutic dose in grams per 60 kilograms as given by J. E. Moore,[8] with the exception of clorarsen for which the dose is that of the largest ampoule commonly administered. Column 6 is the milligrams per 100 grams of body weight calculated from column 5. Column 7 is a therapeutic index, which was calculated by dividing column 6 by column 3. Column 8 is the same index transposed to a basis of "neoarsphenamine = 100" by dividing all of the values by 3 in column 7 and multiplying by 100. From this column it is readily apparent that in the ratios of the maximum therapeutic doses, neoarsphenamine is by far the most effective drug, sulfarsphenamine and arsphenamine next, trisodarsen less effective, and mapharsen and clorarsen much less effective. Similar indexes were calculated, based on the maximum toxic concentraton for marrow cells, and the maximum tolerated dose in grams per kilogram from the same table,[8] but these indexes showed an even greater superiority of neoarsphenamine and inferiority of mapharsen and clorarsen, so are not included in this article.

It is fortunate that neoarsphenamine proves to be the trivalent organic arsenical of choice in the therapy of bacterial infections, since a method of administration[6] to obtain an effective blood level for 50 per cent of the time over a period of 60 days has been developed from a series of blood arsenic determinations. It has been shown to be effective, if followed exactly, in several patients with staphylococcic bacteriemia and subacute bacterial endocarditis. It maintains a more uniform blood level than with the massive intravenous drip technique[9] over a much longer period and yet less arsenic is administered per unit period of time. It does, of course, introduce much more arsenic than the standard clinical group method of treating syphilis but it does not at any time raise the blood level nearly as high as the blood level rises after a single intravenous injection of 0.6 Gm. of neoarsphenamine in a 60 kilogram person.

Comparative analysis in a similar way of the equally effective concentrations of the sulfonamide drugs indicated that 5.0 mg. per 100 c.c. of sulfathiazole is approximately equal in effectiveness to 10 mg. per 100 c.c. of sulfapyridine, 20 mg. per 100 c.c. of sulfadiazine, and 30 mg. per 100 c.c. of sulfanilamide. There were marked individual strain variations in this susceptibility. It is apparent from this that sulfathiazole is the drug of choice among the sulfonamide drugs for therapy of infections due to the organisms listed in Table VI, except when toxic reactions, danger of meningitis, or the presence of meningitis indicates the use of sulfadiazine or sulfapyridine. Sulfanilamide was so much less effective than the other drugs that there seems to be little reason for using it further in the therapy of infections with an organism listed in Table VI. A more detailed presentation of the experimental evidence and the conclusions in regard to each of these organisms listed in Table VI will appear elsewhere in separate papers for each organism. It has already appeared for *Neisseria gonorrhoeae*.[7]

SUMMARY

Analysis of 211 experiments similar to those in Tables I to V on 118 strains of 11 different species of bacteria by the marrow culture technique comparing the

effectiveness of arsenical compounds and the four commonly used sulfonamide drugs is presented. This analysis indicates that in marrow cultures arsenic trioxide and the seven pentavalent organic arsenicals tested are ineffective against all of the species of bacteria studied in therapeutically feasible concentrations. All of the trivalent organic arsenicals ordinarily used in the therapy of syphilis are effective in marrow cultures against the six species of bacteria listed in column 1 of Table VI in concentrations corresponding to about 100 gammas of arsenic per 100 c.c. These trivalent organic arsenical compounds were almost completely ineffective in this concentration against the five species of organisms in column 2 of Table VI. The trivalent organic arsenicals at this concentration were more effective against most strains of the *Staphylococcus aureus, Streptococcus hemolyticus, Streptococcus viridans,* and *Corynebacterium diphtheriae* than the most effective of the sulfonamide drugs in concentrations of 5.0 to 10.0 mg. per 100 c.c. The trivalent organic arsenicals when compared in these concentrations were not as effective as sulfathiazole or sulfadiazine against the *Neisseria gonorrhoeae* and *Hemophilus influenzae* but were more effective than sulfanilamide. When effective at all, the equally effective concentrations of the six more commonly used trivalent organic arsenicals are shown in Table VIII, with the corresponding arsenic concentrations, maximum tolerated therapeutic doses, and a therapeutic index, indicating their relative promise of effectiveness in clinical use against bacterial infections. There was no consistent correlation between the effective concentrations of the trivalent arsenicals and the sulfonamide drugs tested. The equally effective concentrations among the sulfonamide drugs tested are sulfathiazole, 5.0 mg. per 100 c.c.; sulfapyridine, 10 mg. per 100 c.c.; sulfadiazine, 20 mg. per 100 c.c.; and sulfanilamide, 30 mg. per 100 c.c.

The bacteria tested showed marked species and strain differences in susceptibility to the action of the chemotherapeutic agents, as indicated by the size of the inoculum which could be sterilized by a given concentration, as shown in Table VII. The *Streptococcus hemolyticus,* as shown in Table VII, was the most susceptible organism to the action of the trivalent organic arsenicals, and the *Neisseria gonorrhoeae* was most susceptible to the action of the sulfonamide compounds.

CONCLUSIONS

Neoarsphenamine in a concentration which can be maintained 50 per cent of the time for 60 days clinically, if the directions for administration previously published[6] are followed exactly, deserves further trial in the therapy of subacute bacterial endocarditis and bacteriemias, endocarditis, or other serious infections due to the *Staphylococcus aureus, Streptococcus hemolyticus, Hemophilus influenzae, Neisseria gonorrhoeae,* and, possibly, as a local application in diphtheria. Sulfarsphenamine and arsphenamine are somewhat less and mapharsen and clorarsen are much less effective than neoarsphenamine when compared in concentrations attainable clinically.

Sulfathiazole would appear to be the drug of choice among the sulfonamide drugs now available for clinical use, with sulfadiazine to be substituted should sulfathiazole not be tolerated, and sulfapyridine or sulfadiazine to be used if meningitis is present or threatened.

REFERENCES

1a. Osgood, E. E.: Marrow Cultures. A Symposium on the Blood, Madison, 1939, University of Wisconsin Press, pp. 219-241.

b. Osgood, E. E.: Culture of Human Marrow: Studies of the Relative Effectiveness of Neoarsphenamine, Mapharsen, Sulfanilamide, Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole on Infections With Streptococcus Viridans (Alpha Hemolytic Streptococcus), Am. J. M. Sc. **200**: 596, 1940.

c. Osgood, E. E., Joski, Julia, and Brownlee, Inez E.: The Superiority of Neoarsphenamine and Sulfathiazole in the Therapy of Staphylococcus Aureus Infections in Marrow Cultures, Surg., Gynec. & Obst. **71**: 445, 1940.

2. Osgood, E. E.: The Chemotherapy of Staphylococcic Infections, J. Pediat. **17**: 740, 1940.

LeCocq, E.: Use of Neosalvarsan in the Treatment of Acute Osteomyelitis and Blood Stream Infections Caused by the Staphylococcus Aureus, West. J. Surg. **44**: 655, 1936.

LeCocq, J.: Personal communication.

Unpublished data.

3a. Osgood, E. E.: Neoarsphenamine Therapy of Bacterial Infections, Arch. Int. Med. **69**: 746, 1942.

b. Unpublished data.

4. Osgood, E. E., and Brownlee, Inez E.: Culture of Human Marrow: Details of a Simple Method, J. A. M. A. **108**: 1793, 1937.

Osgood, E. E.: The Culture of Human Marrow as an Aid in the Evaluation of Therapeutic Agents, J. Lab. & Clin. Med. **24**: 954, 1939.

5. Osgood, E. E., and Ashworth, Clarice M.: Atlas of Hematology, San Francisco, 1937, J. W. Stacey, Inc., pp. 205-206.

Osgood, E. E.: A Textbook of Laboratory Diagnosis, Philadelphia, 1940, The Blakiston Co., pp. 499-500.

6. References 1b and 3a.

7. Osgood, E. E.: The Comparative Effectiveness of Neoarsphenamine, Sulfathiazole, Sulfadiazine, Sulfapyridine, Sulfanilamide, and Other Sulfonamide Drugs Against Neisseria Gonorrhoeae in Marrow Cultures, Am. J. Syph., Gonor. & Ven. Dis. **26**: 133, 1942.

8. Moore, J. E.: The Modern Treatment of Syphilis, ed. 2, Springfield, 1941, Charles C Thomas, p. 76.

9. Leifer, W., Chargin, L., and Hyman, H. T.: Massive Dose Arsenotherapy of Early Syphilis by Intravenous Drip Method: Recapitulation of the Data (1933 to 1941), J. A. M. A. **117**: 1154, 1941.

THE EVALUATION OF ANTISEPTICS AND OTHER ANTI-INFECTIOUS AGENTS*

WARD J. MACNEAL, M.D., AND NANCY C. FARNSWORTH, B.S.
NEW YORK, N. Y.

COMMUNICABLE diseases and wound infections have assumed an increased interest because of the continuing extension of belligerent activity in the world and the measures for control of these disorders have again become of primary importance in medicine. One group of agents useful for this purpose includes those which may be employed to kill or inactivate disease-producing microbes or to restrict their activity so as to render them wholly or relatively harmless. Although not quite exact in application, the term antiseptics is used in a broad sense to designate the agents in this category and in this sense the germicides are included among the antiseptics.

Antimicrobic agents may be applied in various ways for the control of infectious diseases and, in general, they may be roughly grouped in three classes according to locus of action. Those which are applied to the microbe while it is still at a distance from its possible new victim are in the first category. Methods for sanitary disposal of human wastes, especially incineration, and the adequate protection of water and food supplies belong here. The boiling of drinking water, as in the preparation of tea in China, and the general chlorination of potable waters elsewhere rank high as hygienic measures. By the time the water is actually ingested by man the dangerously destructive high temperature or high concentration of chlorine is no longer present. These agents perform their beneficent function and then become diluted, chemically combined or otherwise vitiated before reaching the human body. If, however, they still retain their death-dealing properties at this time, the possible danger to man requires attention. Food sterilized by heat may be quite acceptable while that preserved with mercuric chloride or formaldehyde is not. Much of our knowledge of these protective measures belongs in the realm of rather ancient history[1] and the practical applications in sanitation have become routine. However, there still remain unsolved problems in this field and further exact information as to the existence and distribution of pathogenic agents outside the human body may lead to new practical advances. The evident distribution of the virus of poliomyelitis in sewage[2] and by con-

*From the Department of Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

Aided by a grant from the Lambert Pharmacal Company, St. Louis, Mo.

Presented in part before the Joint Session, New York City and New Jersey State Branches, Society of American Bacteriologists, New York City, Dec. 29, 1942.

Received for publication, Sept. 24, 1942.

taminated insects[3] and the animal reservoirs of the virus of yellow fever[4] may be mentioned as items presenting new opportunities in this field.

In the second category may be placed those agents and procedures designed to protect from infection by their application to the surfaces of the human body, the skin, mucous membranes or recently contaminated open wounds. Carbolic acid, mercuric chloride, oxidizing agents and various alcoholic preparations are thus used for their local action as distinct from effect after absorption. The revolutionary advances in antiseptic and aseptic surgery which resulted from the epoch-making observations of Lister were initiated by the use of these agents and no adequately informed person could fail to acknowledge their real value when properly employed. These chemical substances are general protoplasmic poisons, able in some instances to destroy human tissues and even to kill the patient if administered internally in sufficient amounts. Hence they are used for their *local action* in such a way that the amount absorbed is too small to induce dangerous poisoning. This fundamental concept, very clearly understood in the preceding century, has become somewhat obscured by the teachings of some modern authors who have failed to distinguish clearly between the *local* protective action of an antiseptic and the effect of an antimicrobic agent *after absorption*. Garrod[5] has expressed his astonishment that this distinction has recently been so lightly disregarded. He has emphasized that local antiseptics help in cleansing the wound, deodorize it, prevent access of further infection and prevent transmission of infection to others; during the first two hours, when the infectious microbes are largely on the surface of a wound (contaminated wound) the local antiseptic may suffice to destroy them. The superficial tissues may be injured or even devitalized and the local leucocytes inactivated by this antiseptic procedure but this local destructive action is temporary and is a small price to pay in consideration of the control of the infectious agent. After a wound has been infected for several hours the microbes may have penetrated well beneath the surface where they cannot be effectively reached by the local antiseptic agent.

In determining the suitability of an antiseptic for local application, the experimental animal with a controlled wound should receive first consideration as a criterion. Russell and Falconer[6] made experimental wounds of the brain in rabbits and inoculated these wounds with streptococci, pneumococci or diphtheria bacilli and then, after lapse of an hour, they applied the antiseptic agent to be tested. Some local injury to the delicate brain tissues was to be expected. However, they succeeded in overcoming the infection and obtained complete recovery of their treated animals in contrast to the fatal outcome in untreated controls, by use of proflavine sulfate and 2:7 diaminoacridine hydrochloride. Such *in vivo* studies of antiseptics require considerable technical skill. Less exacting are many of the tests *in vitro*.

The official standard tests of the Food and Drugs Administration, U. S. Department of Agriculture[7] are relatively more simple. Here only one bacterial organism (*Staphylococcus aureus*) is used and its resistance is checked against carbolic acid. These tests are not considered adequate in all instances

as indicated by the following passage in the officially approved publication[7]: "The limitations of the phenol coefficient make it necessary in some cases to judge the germicidal preparation by other tests or by additional tests." These official procedures do afford evidence to decide whether or not the particular antiseptic is capable of killing or inhibiting the staphylococcus under the standard conditions and these procedures have been extensively employed in testing various antiseptic agents intended for local use.

The practice of using any one simple *in vitro* test as a criterion merits a few words of caution. The point of view is unfortunate. It is much better to make a more complete study of the antimicrobic agent to learn as fully as possible its properties, mode of action, limitations and its possible injurious effects upon vertebrate tissues and, from the results of such studies, to arrive at an estimate of its possibilities for good and harm. Actually one may not justly assume that the behavior of *Staphylococcus aureus* or of any other single species of organism toward an antimicrobic agent is a reliable measure of the behavior of other infectious agents. Furthermore, there is no justification for the assumption that any single technical procedure, *in vitro*, should be accepted as a final criterion for evaluating the antiseptic activity against even the single organism tested. A variation of technique may bring a new revelation. Therefore, new technical procedures should be welcomed as means of extending our knowledge but certainly they should not pretend to supersede the older "standard" methods. If one sets out to devise a single test to extol or to discredit any particular antiseptic, his task is not difficult provided other evidence can be excluded from consideration.

A brief consideration of some common antimicrobic agents may serve to illustrate the pitfalls and to indicate the many factors requiring attention. Mercuric chloride in a 1:1,000 dilution kills within one minute the staphylococcus in a not too heavy watery suspension. However, if the bacteria are suspended in blood or other albuminous fluid, this germicidal effect may be considerably delayed or even prevented. Furthermore, a new element of difficulty is thus encountered because it may not be easy to decide whether or not the microbe has been killed. Transfer to a medium containing ammonium sulfide or *inoculation into the living animal* may reveal continuing viability of the microbe although the ordinary transfer culture may remain inhibited by the associated trace of the germicide. Local tissue injury caused by mercuric chloride may ordinarily appear unimportant but if this agent is applied to large body surfaces for considerable periods it may be absorbed in sufficient amounts so as to induce severe nephritis and fatal anuria.

Iodine, especially in alcoholic solution, has been a favored local antiseptic for many years. It stains the superficial elements of the skin or mucous membrane or wound surfaces, if these are not too moist. The iodine remains for a time in the stained tissue, being more rapidly removed where there is abundant moisture and active circulation of blood. While the tissues are stained with iodine, the bacteria do not grow in them, but by removal of the iodine with thiosulfate one may often discover that the bacteria have not been exterminated

but are only in a dormant state. Hence in testing the germicidal action of iodine, the neglect of this step to inactivate the residual iodine may permit misleading results.

The soaps are only mildly antagonistic to ordinary bacteria but they have long held a favored place in the hygienic care of the skin, accessible mucous membranes and recent superficial wounds, largely because they aid in the mechanical removal of exfoliated epithelium, decomposing secretions, extraneous material and microbes. Practical medical and surgical experience has long justified the confidence placed in soaps. More recently it has been shown[8] that soaps possess remarkable potency to inactivate certain of the disease viruses, such as those which cause influenza, herpes and poliomyelitis. Obviously soaps are used for their local action, and their destructive effect upon blood[9] or internal tissue elements is of academic rather than practical importance in the antiseptic field.

One of the least dangerous of local antiseptics in general use is represented by liquor antisepticus of the National Formulary[10] and the many commercial preparations of similar composition. These are essentially mixtures of dilute alcohol, thymol, boric acid, essential oils and aromatic organic substances, sometimes with added flavoring and coloring matters. Liquor antisepticus is dispensed in a form so adjusted that it should be employed in the undiluted state and when employed as directed it is able to kill staphylococci promptly. Tests against the viruses[11] of cowpox and of influenza have recently shown that these pathogenic agents are quickly inactivated by liquor antisepticus in concentrations above 80 per cent. The germicidal power of this agent is lost when it is diluted to 50 per cent or more and tests performed with such dilutions are likely to mislead.

In the third category of anti-microbic agents we may consider those designed to act *after absorption,* upon microbes which have already penetrated to the internal medium of the living host, where they are surrounded by lymph or blood or by the protoplasm of living cells deep beneath the epithelial surfaces. Previous to the present century this category was made up almost exclusively of biological products, particularly the antisera produced by procedures of immunization, but in addition there were the purified chemicals extracted from cinchona bark, active against malarial parasites, and emetine, active against the dysenteric amoeba. The modern era of enthusiastic chemotherapy in this field began with the dyes, trypan-rot and trypan-blau, which could be given to mice in doses large enough to tint the entire body and, marvelous to tell, in doses adequate to cause, in the living mouse, the sudden and complete disappearance of trypanosomes from the blood, which had been swarming with these parasites before treatment. This achievement of the Ehrlich School was soon followed by the discovery of the arsphenamines, which could be given to rats in doses sufficient to cause a similar sudden disappearance of the spirochetes of relapsing fever previously swarming in the blood. Here, then, were examples of synthetic chemicals capable of selective destruction of certain microbes without, at the same time, killing the animal host. Subsequently various dye substances and many other synthetic chemicals have

been introduced and exploited. The enthusiastic reception of these agents has been due, in part at least, to their demonstrated actual efficiency. The most spectacular of recent antimicrobic chemotherapeutic agents are represented by the sulfonamides. It is possible to introduce sulfanilamide into mice by stomach tube or by parenteral injection in sufficient quantity to overcome an otherwise fatal infection with hemolytic streptococci and abundant clinical trial has demonstrated the value of sulfonamides in human infections with bacteria of this type and also of other types, especially gonococcus, pneumococcus, some types of staphylococcus, colon bacilli and sporogenic anaerobes. The continuing exploitation of new kinds of sulfonamides, which appear to be well-nigh inexhaustible in variety, affords opportunity for continued renewal of enthusiastic expectations and the febrile patient as well as his physician is often tempted to use one of these drugs even before the microbic cause of the fever has been ascertained, sometimes with disastrous result.

In testing these drugs, their administration to animals infected with the particular microbe was the first and still remains the basic and most reliable criterion of efficiency. Testtube methods are less expensive. One may determine the drug concentration necessary to prevent or restrict the growth of the particular bacteria in a culture medium such as broth, agar or citrated blood. When this is carefully done some interesting observations emerge, showing that biological study of this sort is not so simple as might at first be assumed. Prevention of bacterial growth has been found to depend not alone on the concentration of the drug in the medium but also to an important degree upon the size of the bacterial inoculum. Thus, when less than 1000 streptococci have been seeded, their growth has been completely suppressed by sulfanilamide in a concentration of 1 to 100,000, while with an inoculum of a million or more streptococci growth goes forward even in a drug concentration of 1 to 100. Furthermore, it has been found that the addition of a minute amount of para-amino-benzoic acid[12] to the culture medium serves to overcome the bacteriostatic influence of the sulfonamide and thus to permit growth even of a small inoculum. Other substances not yet precisely identified exhibit a similar influence.

Very ingenious technical methods have been employed by Fleming[13] to demonstrate and to test the antibacterial effects of the sulfonamides and of some other antiseptics. He uses slide cells in which citrated blood inoculated with variable small numbers of hemolytic streptococci and mixed with variable small amounts of drug are sealed in with sterile vaseline paper strips and incubated. Bacterial growth is grossly revealed by hemolysis and can be recorded by photography. When freshly prepared, the citrated blood contains living leucocytes and the conditions approximate those of the circulating blood. A small inoculum of less than ten streptococci may be completely suppressed by the natural citrated blood itself without addition of drug, whereas, the same citrated blood after passage through cotton to remove the leucocytes will permit the streptococci to grow. Even more interesting, it was found that the addition of minute amounts of antiseptics, as, for example, carbolic acid 1 part in 700 of blood, serves to inhibit the natural anti-bacterial activity of the

phagocytic cells so that the minute inoculum of streptococci is permitted to grow and to hemolyze the blood while the control preparation without phenol shows no growth. Of course, when a sufficient strength of the phenol is used the growth is suppressed. Fleming has used this method to ascertain the effects in blood of very minute concentrations of various older antiseptics as well as of the newer sulfonamides and also to demonstrate the importance of the size of the inoculum and the variable behaviors of different kinds of bacteria. In another technical procedure, Fleming[13] has used solid agar in a Petri dish. Decimal dilutions of the bacterial culture are spread as broad streaks across the dish on the surface of the medium. Then a segment of the solid agar is cut out at one side of the dish and the resulting defect is filled with melted agar containing the drug to be tested, for example, 1 to 300 sulfanilamide. Diffusion of the drug extends from the insert into the original agar and tends to inhibit bacterial growth to a variable distance from the line of junction and by observation of this inhibition one may estimate the relative susceptibility or resistance of the particular bacterial strain to the drug and may also observe the importance of size of inoculum in determining the growth. This procedure is perhaps less laborious and exacting than the blood-slide culture method and it lends itself to the prompt testing of a culture recently isolated from the patient. These studies of Fleming help toward a better understanding of the action of chemotherapeutic agents in the blood stream and in the internal tissues, *after absorption.*

Somewhat analogous comparative studies of tissue toxicity and antibacterial influence have been carried out by other investigators. Osgood[14] has used *in vitro* tissue cultures of the cells of bone marrow to ascertain the quantity of drug required to inhibit bacterial growth in such cultures and the quantity of drug which will bring about destruction of the tissue cells, thus determining the effective antibacterial concentration and the effective tissue-toxicity concentration.

More recently Welch[9] and his associates have attempted to estimate toxicity of antibacterial agents by microscopic observation of phagocytic activity of leucocytes in diluted citrated blood to which the drug is added and to compare this tissue toxicity with the germicidal effect of the drug in a somewhat different dilute citrated blood.

We have used technical methods of Fleming and those of Welch in the study of various chemotherapeutic agents and have been able to confirm the observations of Fleming in regard to the sulfonamides. By the technic of Welch, however, it was found that azosulfamide is "toxic" in a 1:100 dilution and "not germicidal" in any concentration applicable by the prescribed technique and hence having an immeasurable "toxic index" above 40. Precisely analogous results were obtained with sodium sulfapyridine. Unfortunately, Welch and his associates have attempted to utilize this technical procedure, employing citrated blood, as a criterion for evaluation of antibacterial agents designed for *local use* on the body surfaces, an application which, as has been so clearly indicated by Garrod, is unscientific and likely to be misleading. In

our laboratory a test, by the technique of Welch, of such substances as lemon juice, whiskey, clear tea infusion, black coffee infusion, grapefruit juice, vinegar, tomato juice and baking soda, has shown that each of these agents exercises, according to this technical procedure, a powerful "toxic" inhibitory influence upon the phagocytic activity of leucocytes, while they are without demonstrable germicidal value.

The "toxicity" tests upon tissue cultures or upon fresh citrated blood have real value in the study of antibacterial agents acting *after absorption*. They are not, however, entirely adequate and reliable and may fail completely to disclose the important danger. The animal body includes different kinds of tissue elements which vary among themselves in their reactions to drugs. A concentration of alcohol too weak to influence the cells in a tissue culture or to inhibit leucocytes in citrated blood, may, nevertheless, when present in the circulating blood, cause alterations in the living nervous system which result in traffic accidents. Mercuric chloride in the blood in a concentration too small to influence phagocytosis may cause death from nephritis and uremia. Sulfamethylthiazole maintained in the blood at a level without demonstrable effect on leucocytes or tissue cultures may induce enduring paralysis. Sulfapyridine and sulfathiazole cause toxic injury, not by inhibiting phagocytosis, but by lesions in kidneys, gastrointestinal irritation, changes in bone marrow, myocardial injury[15] and other more obscure alterations. Deaths[16] directly ascribed to administration of the sulfonamides have recently been mentioned in medical publications. To those familiar with the methods of pharmacologic science, these comments will appear superfluous, but they seem to be needed at this time.

In our laboratory an attempt is being made to study tissue toxicity upon the *entire animal* by the relatively inexpensive procedure of injecting the drug into embryonated eggs.[17] The developing chick is a fairly standardized test object and the injected drug does not escape from the shell before hatching. Death of the embryo or survival and successful hatching are grossly evident. The method lends itself to comparative tests of antimicrobic agents alone, of these same agents mixed with infectious viruses or bacteria and of the infectious agents alone. It may prove useful.

SUMMARY

A distinction has been drawn between three groups of antimicrobial agents according to locus of action: (1) those acting apart from the human host called disinfectants; (2) those acting on the epithelial surfaces or upon superficial wounds, designated as antiseptics; and (3) those acting after absorption and classed as anti-infectious therapeutic agents, chemical and biological.

There is no single, easy and simple technical method of evaluating all these agents. The method of Ehrlich and Kolle, in which one administers large amounts to animals to ascertain the lethal dose and smaller amounts to infected animals to ascertain the therapeutic dose, approaches most nearly the ideal procedure. Unfortunately, not all microbes can be tested against drugs

in this way and when feasible the experiments are costly and the relationships sometimes so complex as to leave many questions unanswered. Various kinds of studies, including those *in vitro,* are, therefore, to be welcomed. We are opposed to the attempt to evaluate an anti-infectious agent on the basis of a single "standard" test and we must be on guard against the misleading result of a specially devised technique designed to extol or to condemn some particular agent. It is important also to recall that the causes of infectious diseases are many and varied in nature and that the drug effective against a spirochete may not control the anthrax bacillus, nor may the agent effective against the pneumococcus be assumed necessarily to be potent against the virus of small-pox or influenza. Especially unfortunate is the all too common assumption that an "antiseptic" must be considered worthless unless it may be relied upon to control all infectious agents in all concentrations. So far as we know, only incineration may be accorded this degree of efficiency with full confidence.

Biology is a science of complexities and the control of infectious diseases is a biological problem of the first order. We suggest therefore a broad approach to the study of anti-infectious agents, which welcomes all obtainable information from every reliable means of observation. One may thus determine the peculiar properties of the agent studied and may decide as to its place of usefulness, if any, in the control of infectious diseases. Too frequently the lack of this essential knowledge about an antiseptic and its applicability is the actual reason for failure in its use.

CONCLUSIONS

1. Anti-infectious agents acting on microbes at a distance from the host may be designated as disinfectants. Their toxicity for the human host may be unimportant when it is vitiated before they reach the host.

2. Agents applied to the body surfaces to oppose disease germs may be designated as antiseptics. Such agents should exert the desired local effect upon microbes and should be free from danger of serious poisoning, particularly after absorption. Animal experimentation, tests *in vitro* and clinical trial may contribute to the evaluation of such agents.

3. Antimicrobic substances acting after absorption belong to the category of chemical and biological therapeutic agents. The desired effect upon the microbe should be obtained by a drug concentration much less than that required to kill the animal host. Possible toxic effects may endanger particular organs or tissues and may sometimes be revealed only by animal experimentation or clinical trial.

4. It is possible to discredit or to extol a supposed antiseptic by employing a single selected test procedure to the exclusion of other evidence. Such misleading methods are not recommended.

5. Evaluation of an anti-infectious agent should be based upon all available knowledge of its properties obtainable by various procedures.

REFERENCES

1a. MacNeal, Ward J.: The Preservation of Food. Chapters in Microbiology edited by Charles E. Marshall, Philadelphia, 1917, P. Blakiston's Son and Co.

1b. MacNeal, Ward J.: Pathogenic Micro-organisms, Philadelphia, 1920, P. Blakiston's Son and Co.

2. Paul, John R., Trask, James D., and Gard, Sven: II. Poliomyelitic Virus in Urban Sewage, J. Exper. Med. **71**: 765-777, 1940.

3. Paul, J. R., Trask, J. D., Bishop, M. B., Melnick, J. L., and Casey, A. E.: The Detection of Poliomyelitis Virus in Flies, Science **94**: 395-396, 1941.

4. Bugher, John C., Boshell-Manrique, Jorge, Roca-Garcia, Manuel, and Gilmore, Raymond M.: The Susceptibility to Yellow Fever of the Vertebrates of Eastern Colombia. I. Marsupalia, Am. J. Trop. Med. **21**: 309-333, 1941.

5. Garrod, L. P.: Discussion of the Effect of Antiseptics on Wounds, Proc. Roy. Soc. Med. **33**: 487-494, 1940.

6. Russell, Dorothy S., and Falconer, Murray A.: Discussion of the Effect of Antiseptics on Wounds, Proc. Roy. Soc. Med. **33**: 494-497, 1940.

7. Ruehle, G. L. A., and Brewer, C. M.: United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants. Circular No. 198, U. S. Department of Agriculture, Washington, D. C., December, 1931 (quotation from page 10).

8. Burnet, F. M., and Lush, D.: The Action of Certain Surface-Acting Agents on Viruses, Australian J. Exper. Biol. & M. Sc. **18** (Part 2): 141-150, 1940.

9a. Welch, Henry, and Hunter, Albert C.: Method for Determining the Effect of Chemical Antiseptics on Phagocytosis, Am. J. Pub. Health **30**: 129-137, 1940.

9b. Welch, Henry, and Brewer, Charles M.: Relative Toxicity of Certain Antiseptics Containing Soap and Alcohol. With Special Reference to Mouth Washes, Am. J. Pub. Health **32**: 261-267, 1942.

9c. Welch, Henry, Slocum, Glen G., and Hunter, Albert C.: Method for Determining the Toxicity of Antiseptics as Measured by the Destruction of Human Leukocytes, J. Lab. & Clin. Med. **27**: 1432-1438, 1942.

10. National Formulary, ed. 4, Official from September 1, 1916, American Pharmaceutical Association, Philadelphia, 1918, J. B. Lippincott Co.

11. Dunham, Wolcott B., and MacNeal, Ward J.: Inactivation of Influenza Virus by Mild Antiseptics, J. Bact. **43**: 85, 1942.

12a. Woods, D. D.: Relation of p-amino-benzoic Acid to Mechanism of Action of Sulfanilamide, Brit. J. Exper. Path. **21**: 74-90, 1940.

12b. Wood, W. Barry, and Austrian, Robert: Studies on the Anti-bacterial Action of the Sulfonamide Drugs. II. The Possible Relation of Drug Activity to Substances Other Than p-amino-benzoic Acid, J. Exper. Med. **75**: 383-394, 1942.

13. Fleming, Alexander: Antiseptics and Chemotherapy. Section of Odontology, Proc. Roy. Soc. Med. **33**: 127-136, 1939-1940.

Fleming, Alexander: Discussion of the Effect of Antiseptics on Wounds, Proc. Roy. Soc. Med. **33**: 487-494, 1940.

14a. Osgood, Edwin E.: Culture of Human Marrow: Studies of the Relative Effectiveness of Neoarsphenamine, Mapharsen, Sulfanilamide, Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole on Infections With Streptococcus viridans (Alpha Hemolytic Streptococcus), Am. J. M. Sc. **200**: 596-603, 1940.

14b. Osgood, Edwin E.: Neoarsphenamine Therapy of Bacterial Infections, Arch. Int. Med. **69**: 746-765, 1942.

15. French, A. J., and Weller, C. V.: Interstitial Myocarditis Following the Clinical and Experimental Use of Sulfonamide Drugs, Am. J. Path. **18**: 109-118, 1942.

16. Lederer, Max, and Rosenblatt, Philip: Death During Sulfathiazole Therapy. Pathological and Clinical Observations on Four Cases With Autopsies, J. A. M. A. **119**: 8-18, 1942.

17. Dunham, Wolcott B.: Toxicity of Antiseptics for the Chick Embryo, Proc. Soc. Exper. Biol. & Med. **50**: 274-276, 1942.

SPONTANEOUS RUPTURE OF THE SPLEEN DUE TO ACUTE LEUCEMIA
or
ACUTE LEUCEMIA DUE TO TRAUMA TO THE SPLEEN . . . WHICH?*

Report of Case and Review of the Literature

A. S. Rubnitz, M.D., Omaha, Neb.

With the Collaboration of E. B. Floersch, M.D., Council Bluffs, Iowa

SPONTANEOUS rupture of the spleen in leucemia is a rare occurrence. A review of the literature revealed only seven such possible cases. Hammesfahr[1] (1923) states: "Unusually rare are spontaneous ruptures (of the spleen) in leucemia, of which there are only two cases in the literature, described by Rokitansky (1861)." Lange[2] (1928) mentions five cases from the literature and adds one of his own. Only one more case has been recorded since 1928, that of Neal[3] (1940).

As to the relation of trauma to leucemia, Olovson[4] (1939) collected 67 cases from the literature, and added two of his own, in which leucemia happened to occur in association with trauma to the spleen or to long bones. Six more cases have been reported since Olovson's paper was published: three by Yaguda and Rosenthal[5] (1939), two by Conzett[6] (1939) and one by Schau[7] (1939). There was also one case reported by Novikoff (1933) (cited by Forkner[8]) which Olovson failed to include in his tabulation, so that altogether about 76 such cases have been recorded. While it is true that in many of the reported cases the authors did not claim that trauma was responsible for the leucemia, yet a sizable number of cases have accumulated in the literature in which an etiologic relationship between the two seemed to exist.

The case presented here is of interest primarily because of the rarity of spontaneous rupture of the spleen in leucemia and secondarily because it brings up for consideration the question of traumatic leucemia.

CASE REPORT

H. B., white male, aged 24, milk delivery-man, was admitted to the Mercy Hospital on January 31, 1941. Chief complaints: weakness, abdominal cramps, nausea, profuse sweating and loss of weight. Family history was unimportant. Past history: at the age of 11 he was operated upon for appendicitis complicated by peritonitis. At the age of 13 he suffered a severe injury to the left side of the abdomen and the left flank, in a coasting accident; this was followed by hematuria. The physician who attended him at that time expressed the belief that both the left kidney and the spleen were ruptured. However, the hematuria quickly subsided, and the patient recovered from the accident in a relatively short time, seemingly suffering no ill effects subsequently.

History of present illness: On December 22, 1940, while the patient attempted to climb on the wagon in the course of his daily work, the horse started unexpectedly with a

*From the Department of Medicine, University of Nebraska, Omaha, and the Department of Pathology, Mercy Hospital, Council Bluffs, Iowa.

Received for publication, Sept. 28, 1942.

jerk; the patient missed the step and fell to the pavement twisting his body and the lower back. Upon examination at his home within three hours after the accident, skin abrasions over the right elbow were the only visible marks of injury. The objective findings were those of "lumbo-sacral sprain," and the patient was taped with adhesive. At the physician's office the following day, x-ray examination of the back and pelvis showed no fractures. Patient returned to work in a few days. About a week later he came down with the "flu" (as diagnosed by the attending physician). In a few days he again returned to work, but complained of being easily fatigued. On January 25, 1941, he reported for a physical examination, expressing the belief that he was suffering from "stomach ulcers." A routine blood count was done; the hemoglobin was 75 per cent; the erythrocytes numbered 4,200,000 and the leucocytes 9,800 per c.mm. Examination of the blood smears was not made. He was advised to go to the hospital for observation. This he refused to do at first, but consented to a few days later (Jan. 31).

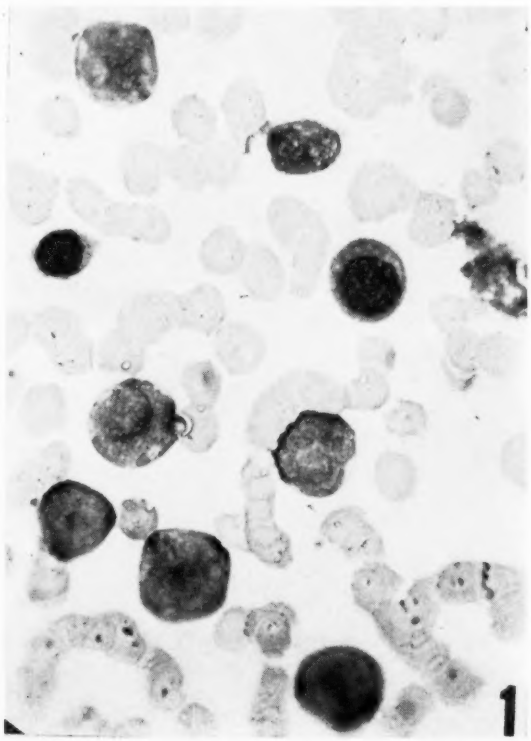


Fig. 1.—Photomicrograph of blood smear taken on Feb. 13. ×930.

Upon entrance to the hospital, his temperature was 102° F.; there was abdominal tenderness and rigidity, and slight cervical adenopathy. The blood count was: Hgb. 14 grams; red blood cells, 4,640,000; white blood cells, 21,500. The differential count (as recorded by the technician) was: segmented neutrophiles 8 per cent; eosinophiles 1 per cent; lymphocytes (?) 91 per cent.

Progress in the Hospital.—The leucocyte count was 35,000 on Feb. 3. A careful study of the blood smears (A. S. R.) disclosed that almost 80 per cent of the leucocytes were promyelocytes, with occasional blast-cells. Most of the cells were oxydase positive. These findings were suggestive of acute leucemia. The clinical picture, however, did not warrant this diagnosis: there were no purpuric manifestations, no ulcerative lesions in the mouth, and but slight swelling in the cervical lymph glands. The patient's chief complaint was abdominal pain, not limited to any particular region. On account of overlying

muscle guard the spleen could not be palpated. The leucocyte count continued to rise: on Feb. 4 it was 40,700; on Feb. 7, 68,400, and on Feb. 11, 82,400. The appearance of the cells in the stained smears was frankly leucemic. In the last smear (see Figs. 1 and 2) 88 per cent of the leucocytes belonged to the young myelocytic series, mainly promyelocytes with occasional blast-cells and rare myelocytes. Nucleated red cells became very frequent. The red count on Feb. 11 was 3,600,000 and the hemoglobin 10.2 grams. A diagnosis of acute leucemia was made and a poor prognosis given. In spite of this the patient did not appear to be critically ill.

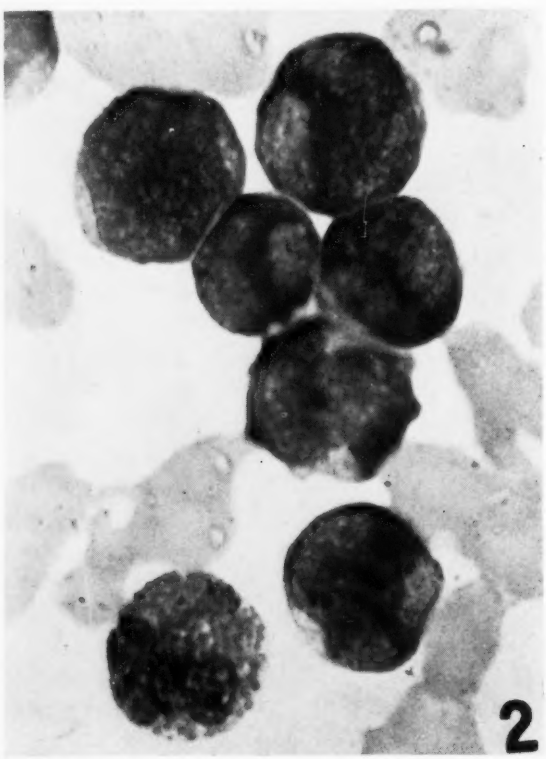


Fig. 2.—Photomicrograph of blood smear taken on Feb. 13. ×1700.

Other laboratory findings: (1) The urine contained a trace of albumin, isolated leucocytes and rare granular casts. (2) The blood N.P.N. was 36 mg. per cent and the creatinine 1.4 mg. per cent. (3) The hetrophile antibody reaction was negative. (4) The icterus index was 5. (5) The blood culture remained sterile at the end of 72 hours.

On Feb. 14 the patient, feeling unable to eat his breakfast, "forced down some food" and vomited shortly after. About 10 A.M. he became very restless and complained of weakness and pain in the precordium, requiring two hypodermic injections of morphine during the day for relief. He dozed for short intervals but continued to complain of precordial pain. His respirations became labored. At 6 P.M. his temperature rose to 104°, his pulse could not be counted accurately (recorded as 156?) and the respirations were 42 per min. He became irrational and involuntary. He died at 3 A.M., Feb. 15.

Autopsy was done at 8 A.M. Feb. 15. The body had been embalmed but the abdominal cavity had not been disturbed.

Important gross findings, as abstracted from the autopsy protocol: The left pleural cavity contained over one quart of thin blood-tinged fluid. The pericardial sac contained about 100 c.c. of bloody fluid. There were some ecchymotic areas in the epicardium near

the apex. The peritoneal cavity contained over one quart of clotted blood. A mass of confluent glands, the size of a hen's egg, was present in the mesentery of the ileum near the cecal juncture. The entire root of the mesentery was infiltrated with large glands, many of them the size of a walnut (see Fig. 4). The retroperitoneal lymph nodes along the aorta were greatly enlarged and formed a continuous chain extending from the pelvis to the diaphragm. The left kidney was rather small, weighing 110 grams; it was round

Fig. 3.—Photograph of ruptured spleen.

Fig. 4.—Photograph of mass of glands in the root of the mesentery.

and flat; the calices were distorted. The right kidney was of normal shape and quite large, weighing 330 grams. The spleen was enveloped in a mass of omentum. As the omentum was shelled off, a very soft, enlarged ruptured spleen was found. The rupture occurred almost through its middle, transverse to the long axis, and extended from the concave border nearly halfway across the width of the organ (see Fig. 3). The spleen weighed 420

Fig. 5.—Photomicrograph of section of the spleen. ×140.

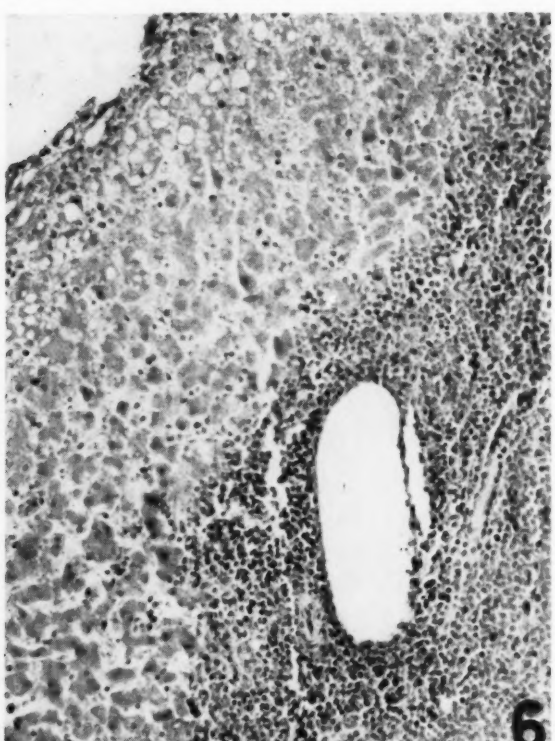


Fig. 6.—Photomicrograph of section of the liver. ×140.

grams; the tissue was unusually soft. On section, the color varied from reddish-brown to dark-chocolate. Many areas were cystic, the tissue being in a fluid state. The liver weighed 2390 grams. The cut surface was very soft. Both lungs had dark patches at the bases. On section, large edematous areas were present through the posterior portions of both lungs, especially in the lower lobes.

Summary of the gross anatomic findings: (1) Ruptured, degenerated spleen; (2) massive intraperitoneal hemorrhage; (3) swelling of the mesenteric and retroperitoneal lymph nodes; (4) left hydrothorax; (5) hemopericardium; (6) distortion and scarring of the left kidney and compensatory hypertrophy of the right kidney; (7) hypertrophy and degeneration of the liver; (8) pulmonary edema.

Important microscopic findings: Sections of the lymph nodes presented the usual histologic picture of leucemia. Frank leucemic changes were also evident in sections of the spleen, though here the process of necrosis overshadowed all other findings (see Fig. 5). Sections of the liver showed generalized parenchymal necrosis, fatty metamorphosis, and areas of leucemic infiltration. The latter were most pronounced in the interlobular connective tissue, around the ducts (see Fig. 6). Leucemic infiltration was also apparent in sections of the pancreas, kidneys, adrenals and lungs.

COMMENT ON CASE

It is difficult to evaluate the history of injury at the age of 13. According to the physician who attended him at that time, the patient had a hematuria which cleared up in a few days. Rupture of the spleen was suspected at first but the patient seemed to be in good physical condition and never manifested any evidence of internal hemorrhage. Moreover, by the time the hematuria disappeared, he seemed to have totally recovered from the effects of the injury.

The history of the accident six weeks before the patient's entry to the hospital permits a good deal of speculative reasoning.

One could assume that the patient suffered a subcapsular tear in the spleen, and that a hematoma was formed. The patient continued to complain of abdominal discomfort and developed fever and malaise which was diagnosed as "the flu." The hematoma could lead to increased tension within the spleen and cause degenerative changes in the pulp. All through the period of illness the degeneration might have continued to progress, and finally the organ might have ruptured from a trivial increase in pressure when the patient turned in bed or during the act of vomiting. This line of reasoning does not take into account the leucemic changes in the blood and tissues.

Again, one might speculate that the accident had nothing to do with the patient's illness. The onset of the illness might have happened to coincide with the time he returned to work after the accident. His complaints of malaise, weakness, anorexia and abdominal cramps were diagnosed as "the flu," a diagnosis often made at the onset of acute leucemia. The blood findings were not characteristically diagnostic at first but became more and more so as the disease progressed, simultaneously with the rapidly changing blood picture, and culminated in sudden spontaneous rupture.

A third combination could be rationalized: it might be assumed that the recent accident was not severe enough to produce rupture in a normal spleen. However, the accident suffered by the patient at the age of 13 might have produced a tear in the spleen which had healed. The scar formed in the line of healing might have given way during the last accident and brought about all

the sequelae of the first mentioned possibility. The fact that the spleen was highly degenerated made it impossible to demonstrate any preexisting lesions in this organ. From the appearance of the left kidney, however, one could surmise that the distortion in its shape was due to the coasting accident and that the spleen quite likely also suffered injury at that time.

GENERAL DISCUSSION

Spontaneous rupture of the spleen is invariably associated with preexisting disease of this organ. A normal spleen will not rupture unless it is subjected to violence of a considerable degree. Rupture of the spleen in the absence of obvious trauma is an indication that deep-seated degenerative and necrotic changes have taken place. Necroses of the spleen in leucemia are not uncommon. According to Lubarsch (cited by Schultz[9]), they occur in up to 50 per cent of the cases of myeloid leucemia, and are supposedly due to arterial thromboses. The damage of the arterial wall may be similar to that produced in periarteritis nodosa which leads to the formation of thrombi. According to Benda (cited by Schultz), the infiltration of the vascular wall with leucemic cells is a common occurrence in leucemia. Such infiltration within the walls favors formation of thrombi within the lumen. Another factor, initiating thrombus formation in leucemia, was pointed out by Hirschfild (cited by Schultz), as being the increased viscosity of leucemic blood. He explained the formation of thrombi in the corpora cavernosa on the same basis.

While the above-mentioned vascular changes produce or tend to produce necrotic changes in the spleen, such necroses are generally of a mild degree. Quite rare are the necroses which involve the greater part of the organ, as in the case recorded by Schultz. Even in his case the necrosis did not lead to rupture of the spleen. Our case is unique in that the degeneration of the spleen involved almost the entire organ. Such extreme necrosis has been recorded by Schtscherbatoff (cited by Friesleben[10]) in a patient who was recovering from a severe case of typhoid fever and who after a very light strain suddenly collapsed and died. Autopsy disclosed a transverse rupture of a totally disintegrated spleen.

The sequence of events in our case could be viewed from two different angles: (1) This was a case of rapidly progressing acute leucemia. The degenerative changes in the spleen paralleled the day-by-day hematologic changes, culminating in total disintegration and rupture of the organ. (2) This was primarily a case of degeneration of the spleen, initiated by a subcapsular hematoma of traumatic origin. The leucemic changes in the blood resulted from abnormal stimulation of the hematopoietic apparatus by the disintegrating spleen.

In a paper published under the title "Leukemia Following Trauma," Lewsen (1930)[11] collected sixty cases from the literature, including one case of his own. However, the evidence in twenty of the cases was not convincing and he tabulated only 40 of the cases (all of these are also included in Olovson's tabulation). The type of trauma was classified by Lewsen into three groups: (1) blow to long bones or ribs which may or may not result in a fracture; (2) blow in the region of the spleen, sometimes rupturing that organ, and (3) a general severe shaking. Lewsen concedes that trauma to

hematopoietic tissue does not always result in leucemia. He is of the opinion that leucemia would be produced only in individuals who have an inherent predisposition to this disease. He draws an analogy between traumatic leucemia and traumatic sarcoma, pointing out that trauma alone, without the individual predisposition, would hardly be sufficient cause for the production of either.

Analyzing his own tabulated cases, Olovson concludes that they could be divided into 3 groups: (1) those in which trauma leads to the discovery of an existing leucemia; (2) those where trauma causes an aggravation of an existing leucemia, and (3) those where trauma seemingly causes the leucemia. It is obvious that our attention would be centered principally on the cases of group three. Yet, even in these, according to Olovson, trauma could play but a minor role in the production of the disease, because traumata occur frequently whereas traumatic leucemia is a rare occurrence. He is of the opinion that trauma may constitute the exciting factor in a person predisposed to leucemia.

Yuguda and Rosenthal are still more outspoken in their view that trauma could not be the sole factor in the production of leucemia. These authors believe "that trauma initiates leucemia in a person with a preexisting tendency to the disease. The injury upsets the fine hematopoietic balance which has maintained the leucemia in its latent state and causes a more rapid progression of the process with development of frank symptoms of leucemia."

In the main all the above authors agree that trauma to hematopoietic tissue by itself could not be considered sufficient cause to produce leucemia. They all believe that trauma plays the role of a precipitating factor, in an individual who has an inherent tendency to the disease.

Mention may be made here that the problem of trauma and leucemia also attracted attention in veterinary medicine. Cases of leucemia following trauma have been observed in a sheep, a horse, and in dogs, as reported by Weber, Wirth, Share-Jones and Jarmai (all these authors are cited by Olovson). Jarmai conducted a series of experiments on dogs in which he traumatized the spleen by an open operation. However, all the experiments resulted in failure to produce any leucemic changes in the animals.

The problem of trauma and leucemia is of special importance from the medicolegal standpoint. In Olovson's series, 19 of the cases have been scrutinized by insurance commissions and 12 were granted compensation. This presumably implies that on the surface, at least, a cause-and-effect relationship seemingly was present in the 12 cases. Furthermore, we should be mindful of the fact that insurance companies will often tend toward leniency in settling an accident claim, particularly where it is known that the individual's illness dated to the accident. None-the-less, insurance companies will not grant compensation unless the case merits consideration.

In order that a case of leucemia could justly be considered as one due to trauma and qualify for compensation, the following criteria were set up by Liniger (cited by Yaguda and Rosenthal):

1. The injured individual must have been well and feeling capable of work up to the time of the accident.

2. A suitably severe accident must have taken place, with the essential subjective and objective signs of injury.

3. The time at which the development of sickness starts and that of the accident must bear a relation, that is, there must be bridging symptoms.

Olovson's criteria are:

1. Good health and absence of disease-symptoms before the trauma.

2. A free interval between the trauma and the first appearance of the symptoms.

3. Leucemia of the myeloid type.

Points 1 in both sets of criteria are essentially identical. Liniger's point 2 does not need any elaboration. Both Liniger's point 3 and Olovson's point 2 deal with the time-element between the trauma and the first manifestations of the disease. However, each one of these authors expresses a different conception and stresses a different point. Both these points merit consideration and can best be emphasized by illustrative cases:

Illustration 1. (Case 2 of Conzette.) A middle-aged woman suffered an intertrochanteric fracture. Upon admission to the hospital, immediately after the accident, her leucocyte count was 188,000 and the blood smear showed the characteristics of chronic myeloid leucemia. The spleen was decidedly enlarged.

Illustration 2. (Case 1 of Olovson.) A 27-year-old male suffered fracture of the leg. The fracture healed and the patient was seemingly in very good health for over 20 months, when he developed pain and swelling at the site of the fracture. At this time his leucocyte count was 347,000 and the blood smear was characteristic of chronic myeloid leucemia.

Of these two illustrations, the first depicts an instance where a leucemic individual suffered a fracture. Olovson's point 2, calling for a free interval between the trauma and the first appearance of the leucemic symptoms, excludes this case from the category of traumatic leucemias, and the case well illustrates the soundness of that point. In the second illustrative case an interval of over 20 months elapsed between the injury and the appearance of leucemic symptoms. There was a total lack of bridging symptoms. Liniger's point 3 would tend to exclude this case from the category of traumatic leucemias.

That the leucemia must be of the myeloid type in order to be considered as of traumatic origin (as advocated by Olovson's point 3) is a strongly debatable question. Nearly all of Olovson's 69 tabulated cases were leucemias of the myeloid type (only 3 of the cases were lymphatic leucemias). These figures, perhaps, were partly responsible for that contention. However, this statistical preponderance of myeloid leucemias is lessened by the fact that out of the 7 cases not included in Olovson's tables, 4 were lymphatic leucemias (one case of Novikoff, one of Schau, one of Yaguda and Rosenthal, and one of Conzette). Discarding the statistical reason for that contention, we come to the more valid reason, namely, that if traumatic leucemia is produced by injury to long bones or to the spleen the implication would be that such injury upsets the myelopoietic tissue, hence leucemia of the myeloid variety only could result. This reasoning is based on the assumption that lymphatic leucemia has its origin in histo-biologic disturbances of the lymphocytic apparatus, whereas myeloid leucemia has its origin in the myeloid tissue. While it is

true that this strict subdivision could be visualized in chronic leucemias, the site of the hematopoietic disturbance in acute leucemias is not as a rule so clearly delimited. The process of metaplasia is a common characteristic of acute leucemia, and tissues which are in no way concerned with the function of hematopoiesis in postnatal life generally revert to their embryonal state and resume such a function, producing abnormal leucocytes of every variety; hence the difficulty in most instances to classify acute leucemias according to cell types. Incidentally, an analysis of all the reported cases of traumatic leucemia reveals that the most authentic cases are those in which acute leucemia resulted from the trauma.

The most weighty argument, however, against Olovson's point 3 is the case reported by Schau:

> An 18-year-old boy was hit in the left side of the abdomen, playing football. At the hospital, rupture of the spleen was suspected but the patient was treated conservatively. The leucocyte count was 9,000. About 7 weeks later the patient was readmitted with a clinical and hematologic picture of frank leucemia; the leucemia, according to the author, was lymphatic in type. Autopsy disclosed an old tear in the spleen.

According to advocated criteria, this case is an excellent example of traumatic leucemia, though it was not one of the myeloid variety.

From the foregoing discussion one may deduct that the criteria advanced by Liniger and by Olovson for the medicolegal evaluation of a case of traumatic leucemia are logical and sound, except point 3 of Olovson in which the author insists that the leucemia must be of the myeloid type.

It might be of interest to mention here that a monetary settlement was granted by the insurance company in our case.

SUMMARY

A rapidly progressing case of acute leucemia is reported. The rapidity of the progress of the disease was most noticeable in the blood changes, whereas clinically the patient did not appear to be critically ill until almost the end. On the fifteenth day of the patient's stay in the hospital his condition suddenly changed for the worse, and after a stormy 18 hour period he expired. Autopsy disclosed a massive intraperitoneal hemorrhage resulting from a ruptured spleen. The spleen was degenerated throughout. The degeneration was attributed to leucemic changes in the organ. However, two previous episodes in the patient's history could have accounted for some damage to his spleen: (1) about 11 years previously the patient was in a coasting accident suffering injury to the left kidney and presumably also to the spleen, and (2) about 8 weeks before his death the patient fell on the pavement, spraining his back and "wrenching" his body. That the patient actually suffered serious injury to the spleen in either of these accidents is only conjectural. Because of the extreme disintegration of the spleen, histologic evidence could not be relied upon to prove or disprove that the organ had suffered injury in the past. It is conceivable that at the time of the coasting accident a tear in the spleen had been produced and that this healed by scar formation. Furthermore, it is conceivable that during the last accident the pulp gave way in the line of the old scar and a subcapsular hematoma was formed, leading to sub-

sequent "spontaneous" rupture. It is granted that no absolute proof exists that the spleen was severely injured in either of the two episodes; yet, because of the fact that the history of the last accident was so intimately tied up with the patient's last illness and because of the patient's persistent complaints of abdominal pain throughout the period of the illness, credence may be given to the view that the degenerative changes in the spleen set in before the hematologic changes were noticeable. Since a number of leucemic cases are recorded in the literature in which trauma to the long bones or to the spleen preceded the leucemic changes, some authors express the belief that leucemia may be precipitated by trauma in susceptible individuals. If we accept the dictum that there is such an entity as traumatic leucemia, our case would fall into that classification, and the entire clinical behavior of the patient, from the day of the accident to the hour of the fatal termination, would appear to be in logical sequence.

The question of the possible causal relationship between the trauma and leucemia is of interest from two angles: (1) the theoretical soundness of the view that leucemia could be caused by trauma, and (2) the medicolegal side of the problem, i.e., should an employer or insurer be held liable for the leucemia in a case where leucemia follows trauma. An attempt has been made in this paper to analyze both phases of the question and to bring out the more authoritative statements and data from the literature regarding this question.

CONCLUSIONS

1. Spontaneous rupture of the spleen in a case of acute leucemia is reported.

2. Since there was a history of an accident suffered by the patient about 6 weeks before the leucemia was established, the case is also discussed as one of possible traumatic leucemia.

3. The literature on the subject of traumatic leucemia is reviewed.

4. The medicolegal criteria recommended for the acceptance of a case as one of traumatic leucemia are scrutinized.

REFERENCES

1. Hammesfahr: Spontanruptur einer leukämischen Milz, Zentralbl. für Chir. **50**: 1634, 1923.
2. Lange, W.: Spontanrupturen der Milz bei Leukämie, Med. Klin. **24**: 1311, 1928.
3. Neal, J. M.: Rupture of the Spleen in Myelogenous Leukemia, M. Bull. Vet. Admin. **17**: 96, 1940.
4. Olovson, T.: Trauma und Leukämie, Acta chir. Scandinav. **82**: 63, 1939.
5. Yaguda, A., and Rosenthal, N.: The Relation of Trauma to Leukemia, Am. J. Clin. Path. **9**: 311, 1939.
6. Conzett, D. C.: Leukemia Associated With Trauma, J. Iowa M. Soc. **29**: 458, 1939.
7. Schau, H.: Lymphatische Leukämie und Milzverletzung, Zentralbl. f. Chir. **66**: 1609, 1939.
8. Forkner, C. E.: Leukemia and Allied Disorders, New York, 1938, The Macmillan Co.
9. Schulz, W.: Ueber nicht-embolisch entstandene keilförmige Milznekrosen, Frankfurt. Ztschr. f. Path. **37**: 489, 1929.
10. Friesleben, M.: Ein durch Splenektomie geheilter seltner Fall von Spontanruptur der Milz, Deutsche Ztschr. f. Chir. **173**: 45, 1922.
11. Lewsen, S. C.: Leukemia Following Trauma, Lancet **1**: 288, 1930.

CLINICAL CHEMISTRY

THE EFFECT OF DIATHERMY ON BRAIN METABOLISM: CHANGES PRODUCED ON THE SUGAR, LACTIC ACID, AND pH OF THE ARTERIAL AND VENOUS BLOOD OF THE BRAIN IN PARETIC PATIENTS

JOSEPH M. LOONEY, M.D., AND EMBRIE J. BORKOVIC, M.D., WORCESTER, MASS.

IN A recent communication[1] we pointed out that during diathermy therapy in our patients the oxygen A-V difference showed a fall rather than a rise as obtained by Himwich and his co-workers.[2] In this paper we are reporting the results of hyperthermia on the sugar, lactic acid, and pH of arterial and venous blood. The same procedure for obtaining blood samples was followed as given in the previous paper.[1]

The blood sugar was determined by the method of Folin,[3] the lactic acid by the method of Edwards,[4] and the pH of the whole blood at 38° C. by the glass electrode and an electron tube potentiometer built according to the method of Stadie.[5] All determinations except the blood sugar were made in duplicate. The means and standard deviations for the different variables for the three periods of observations are given in Table I. Each value represents the results obtained from twenty-one separate experiments.

Both the arterial and venous blood sugar levels showed a fall during the period in which the temperature was rising and then an increase to a higher level than the control reading. The decreases in sugar for both venous and arterial blood, as well as the subsequent increases, were statistically highly significant. The difference between the final and initial readings, however, was not significant for either the arterial or the venous blood. The difference between arterial and venous blood sugar increased from a mean of 7.3 mg. for the first reading to 11.0 mg. for the final reading. This change was not statistically significant, as the P value calculated for this change is 0.32, which indicates that this change could occur by chance 32 times in every 100 trials.

The lactic acid levels in both arterial and venous blood showed a slight decrease during the period of elevated temperature. The decrease was greater in the arterial blood, with a fall from 14.22 to 11.97 mg., than in the venous blood, which decreased from 13.36 to 12.15 mg. and then rose to 12.61 mg. As a result of the difference in the degree of change, the final level of lactic acid in venous blood became higher than that of the arterial blood. Although these changes were not statistically significant, the trend is important when considered in connection with the changes which occurred in the oxygen con-

From the Memorial Foundation for Neuro-Endocrine Research and the Medical Service of the Worcester State Hospital, Worcester, Mass.

Received for publication, Oct. 22, 1942.

TABLE I

MEANS AND STANDARD DEVIATIONS FOR ALL VARIABLES FOR THREE PERIODS

		FIRST READING*		SECOND READING*		THIRD READING*	
		MEAN	S. D.	MEAN	S. D.	MEAN	S. D.
Temperature ° F.		98.41	0.700	105.95	0.389	105.92	0.358
Total serum protein (Gm. per 100 c.c.)	Venous	6.60	0.92	6.97	1.00	7.58	1.18
Blood hematocrit	Venous	40.01	3.53	41.42	4.19	43.04	5.42
Specific gravity	Venous	1.0258	0.00174	1.0267	0.00172	1.0283	0.00165
Blood sugar (mg. per 100 c.c.)	Arterial	126.0	34.38	103.8	22.95	140.3	38.95
	Venous	118.8	30.48	94.0	11.29	132.1	37.33
	A-V† difference	7.3	11.43	9.8	16.14	11.0	10.75
Lactic acid (mg. per 100 c.c.)	Arterial	14.22	4.48	12.39	5.08	11.97	4.26
	Venous	13.36	3.88	12.15	5.65	12.61	4.20
	A-V	0.86	2.61	0.24	5.54	−0.64	2.32
pH	Arterial	7.462	0.066	7.514	0.077	7.497	0.072
	Venous	7.398	0.059	7.459	0.068	7.465	0.067
	A-V	0.064	0.033	0.055	0.026	0.037	0.023

*First reading immediately before diathermy; second reading when temperatures reached 106° F., approximately two and one-half hours later; third reading, after three to four hours' fever at 106° F.

sumption and sugar metabolism. In our previous paper[1] it was shown that the oxygen consumed decreased as the temperature was maintained at the higher level. This would indicate that the rate of combustion in the brain did not increase with the temperature. The slight increase in utilization of sugar could be explained, at least in part, by the transformation of some of the sugar to lactic acid. The values of lactic acid in the arterial and venous blood of the brain for the first two readings indicate the utilization of some lactic acid by the brain tissues during this period. During the last period, however, there is a reversal, and a production of lactic acid occurs, the venous level being higher than the arterial level.

The pH of the blood showed a significant rise during the period of elevated temperature in both venous and arterial blood. In the arterial blood there was an increase from 7.462 ± 0.0148 to 7.514 ± 0.0172 and then a drop to 7.497 ± 0.0162. The difference between the first and last readings was not statistically significant. In the venous blood the changes from the initial reading, 7.398 ± 0.0132 to 7.459 ± 0.0153 for the second reading and 7.465 ± 0.0152 for the final reading, were highly significant. The values of Fisher's P for the differences between the readings were 0.0023 in the first case and 0.0008 in the second.

The change in the arterial-venous differences in the pH values was highly significant (P = 0.002) between the initial reading of 0.064 ± 0.0072 and the final reading 0.037 ± 0.0051 but was not significant between the first and second readings. With the increase in temperature the difference between venous and arterial blood became smaller, the greatest change occurring between the second and the third readings. The fall in the A-V difference between these readings was entirely accounted for by the fall in the pH of the arterial blood.

DISCUSSION

The elevation in blood sugar has been noted by previous investigators.[6, 7] Our results agree with those of Hench,[6] that the elevation is not due to blood concentration, for we obtained a 40 per cent increase in sugar from the lowest to the highest readings and only a 7.5 per cent hemoconcentration on the basis of the hematocrit readings, or a 14.8 per cent increase on the basis of the total protein levels.

Despite this marked increase in the concentration of sugar in the cerebral circulation, there was no increase in the utilization of sugar. The slight increase in A-V difference noted during the third period could be accounted for, in part, by conversion to lactic acid. These results confirm our previous findings[1] that hyperthermia resulting from diathermy therapy does not increase the rate of brain metabolism.

Our findings also lend support to the view that under certain conditions the brain does utilize lactic acid. However, when the temperature is elevated, lactic acid is produced. This may be due to an interference with the delicately balanced enzyme systems as the result of the increased temperature.

The alkalosis produced (amounting to 0.07 pH units in the venous blood and 0.03 pH units in the arterial blood) is due to the loss of carbon dioxide and is not as marked as that reported by Bischoff and co-workers.[8] These

investigators found increases in plasma pH of 0.12, 0.09, 0.26, and 0.04 in four cases. Cullen, Weir, and Cook[9] in a recent paper indicate pH changes of 2.0 pH units. This value is so high that it seems likely that an error has been made in reporting and that the change should be 0.2 pH units. Their results were obtained on blood taken from the arm, and therefore conclusions as to changes which occur in the cerebral circulation are not warranted.

In the case of one of the patients who died following treatment, the blood sugar showed wide fluctuations in level but no difference between the arterial and venous readings, which were 211 and 211, 95 and 94, and 155 and 154 mg. per 100 c.c. One patient showed a remarkable increase in arterial blood sugar with the following values: 98, 109, and 290 mg. per 100 c.c. It would appear that in this case the continued elevation of temperature for the three-hour interval at 106° F. had caused a marked stimulation of the adrenal mechanism and a release of sugar from the glycogen deposits in the liver. In his second treatment the patient showed an elevation from 95 to 164 mg. Another patient showed a rise from 98 to 190 mg. It is evident that although slight increases in sugar are found in all patients during periods of temperature elevation, very marked changes are the exception. The high mean values for sugar found in the control period are due to the inclusion of two men with abnormally high fasting values. During the period in which active heating of the body was taking place, in fourteen of twenty-one tests the blood sugar fell. This fall also occurred in the two cases that had high initial values, in the first instance from 211 to 95 mg. per 100 c.c., and in the second, from 176 to 78 mg. per 100 c.c. This initial fall in sugar has not, to our knowledge, been reported before.

TABLE II

COEFFICIENTS AND PROBABILITIES FOR CORRELATIONS BETWEEN TEMPERATURE AND INDIVIDUAL VARIABLES

CHANGE IN TEMPERATURE VS.	NO. OF READINGS	R	P
Change in venous blood sugar	38	0.7187	$< .01$
Change in arterial blood sugar	37	0.5371	$< .01$
Change in total protein	38	−0.1628	
Change in blood hematocrit	32	−0.0979	
Change in specific gravity	37	−0.2457	
Change in venous lactic acid	38	−0.1516	
Change in arterial lactic acid	38	−0.1119	
Change in venous pH	36	0.5100	$< .01$
Change in arterial pH	36	0.4725	$< .01$

The correlations between change in temperature and the other variables are shown in Table II. It will be noted that only those between temperature and sugar and temperature and pH can be considered significant. Contrary to expectation, the correlations for total protein, blood hematocrit, and specific gravity are very low. Incidentally, the correlation between specific gravity and total protein is also low, as pointed out in a paper published recently.[10]

SUMMARY

The changes produced in cerebral blood sugar, lactic acid, and pH by elevation of temperature through diathermy in twelve patients receiving dia-

thermy treatments are given. There was a slight but not significant increase in sugar utilization and a significant increase in pH. The lactic acid A-V difference becomes reversed so that during the period of continued temperature elevation, lactic acid is produced. This would explain, in part, the slight increase in the A-V difference for blood sugar. The findings confirm those previously reported, that there is no increase in brain metabolism during diathermy.

REFERENCES

1. Looney, J. M., and Borkovic, E. J.: Changes Produced on Oxygen and Carbon Dioxide Content of Arterial and Venous Blood of Brain During Diathermy Therapy for General Paresis, Am. J. Physiol. **136:** 177, 1942.
2. Himwich, H. E., Bowman, K. M., Goldfarb, W., and Fazekas, J. F.: Cerebral Metabolism During Fever, Science **90:** 398, 1939.
3. Folin, O.: The Determination of Blood Sugar, J. Biol. Chem. **82:** 83, 1929.
4. Edwards, H. T.: Simplified Estimation of Lactate in Normal Human Blood, J. Biol. Chem. **125:** 571, 1938.
5. Stadie, W. C., O'Brien, H., and Laug, E. P.: Determination of the pH of Serum at 38° With the Glass Electrode and an Improved Electron Tube Potentiometer, J. Biol. Chem. **91:** 243, 1931.
6. Hench, P. S., and Slocumb, C. H.: Results of Fever Therapy for Gonorrheal Arthritis, Chronic Infectious (Atrophic) Arthritis and Other Forms of "Rheumatism," Proc. Staff Meet., Mayo Clin. **10:** 202, 1935.
7. Kerstein, M. B., and Bromberg, L.: Effect of Fever Therapy Upon Carbohydrate Metabolism, J. LAB. & CLIN. MED. **25:** 7, 1939.
8. Bischoff, F., Long, M. L., and Hill, E.: Studies in Hyperthermia. II. The Acid-Base Equilibrium in Hyperthermia Induced by Short Radio Waves, J. Biol. Chem. **90:** 321, 1931.
9. Cullen, S. C., Weir, E. F., and Cook, E.: Rationale of Oxygen Therapy During Fever Therapy, Anesthesiology **3:** 123, 1942.
10. Looney, J. M.: The Relation Between Specific Gravity of Blood Serum and Its Protein Concentration, J. LAB. & CLIN. MED. **27:** 1463, 1942.

CIRRHOSIS OF THE LIVER: THE LIPOTROPIC ACTION OF PARENTERALLY ADMINISTERED AMINO ACIDS

I. DONALD FAGIN,* M.D., MELVILLE SAHYUN,† PH.D., AND RALPH W. PAGEL,* M.D.
DETROIT, MICH.

IN A recent publication[1] an investigation of the possible therapeutic effects of intravenously administered amino acids‡ in five patients with cirrhosis of the liver was reported. Briefly, the results were (1) subjective improvement of great or moderate degree in four of the five patients during the one month period when the amino acids were used; (2) shrinkage in size of the liver and spleen in all patients in whom these organs were enlarged; (3) disappearance of peripheral edema; (4) indications of improvement in hepatic function, as gauged by hippuric acid synthesis and glucose tolerance tests in all cases, and improvement in bromsulfalein excretion in one case. On the basis of published experimental data it was postulated that the decrease in size of the liver was

*Medical Service and Laboratories, U. S. Marine Hospital, Detroit, Mich.
†Division of Biochemical Research, Frederick Stearns & Company, Detroit, Mich.
‡Fortified acid hydrolysate of casein supplied through the courtesy of Frederick Stearns & Company.
Received for publication, Dec. 14, 1942.

due to the resorption of fat through the lipotropic action of the amino acids. The decrease in splenic size was interpreted as resulting from a decongestion secondary to improved portal circulation.

This report presents data derived from analyses for total lipid and total nitrogen content of the livers of three groups of patients: (1) those who died with cirrhosis of the liver but received amino acid therapy; (2) patients who died with cirrhosis of the liver who did not receive amino acid therapy; and (3) patients who died from other causes without cirrhosis of the liver. The value of this study arises through an opportunity to compare data collected from the chemical study of human livers with those published from similar studies in experimental animals.

Of the five patients who received parenteral amino acids, three (G. R., A. G., and P. W.*) evidenced sufficient clinical improvement to warrant discharge from the hospital. The remaining two patients (J. R. and G. B.*) maintained a clinical plateau for two and one-half and three and one-half months, respectively, following the month of amino acid administration, then entered a rapid and progressive downhill course and died in the hospital. All five patients exhibited a very slight increase in liver size when the administration of amino acids was discontinued; however, in no case did the liver return to its original degree of enlargement, presumably because the patients were maintained on an adequate diet.

To the patients studied and reported, we have added E. C., a patient with chronic alcoholism and advanced "decompensated" cirrhosis of the liver, who died two months after having received amino acids. Autopsies were performed on the patients who died in the hospital (G. B., J. R., and E. C.), and the livers grossly and microscopically exhibited the characteristic pathologic features of portal cirrhosis of the Laënnec or "alcoholic" type. All three patients had a background of chronic alcoholism.

MATERIAL AND METHOD

Formalin-fixed specimens of liver obtained at autopsy from three groups of patients were analyzed for total fat and total nitrogen content; the duration of formalin fixation of the specimens varied from a few days to eighteen months.

Group I consisted of the three patients described above who had cirrhosis of the liver and chronic alcoholism and received parenteral amino acids for three weeks (E. C.) or one month (J. R. and G. B.) during their course.

Group II consisted of patients who had cirrhosis of the liver and chronic alcoholism but had not received amino acids. We reviewed our autopsy protocols for the eighteen-month period prior to this study and found four such cases. No cases with cirrhosis of the liver and chronic alcoholism other than the seven included in Group I and II came to autopsy during this eighteen-month period.

Group III consisted of ten patients who were unselected except that none presented post-mortem evidence of primary liver disease; this group served as a control.

*Abstracts of the clinical records of these patients may be found in Reference 1.

The liver specimens, preserved in 4 per cent formaldehyde, from these three groups of patients were analyzed by the biochemist for total liver fat and total liver nitrogen. These specimens were identified to the biochemist by number only. Glycogen determinations were not done. The analyses for fat were performed, with minor modifications, by the chloroform extraction method used by Channon, Platt, and Smith.[2]

The effect of formalin fixation on liver lipids was investigated by Halliday,[3] who found that, although total solids and total cholesterol content were not significantly affected by treatment with formalin, the total fatty acid content decreased. The decrease was only slight during the first three months of formalin fixation but was considerable after longer periods, probably because of auto-oxidation. Weil,[4] working with nervous tissue, found that phosphatides were hydrolyzed after formalin treatment, but cholesterol and galactolipids were not appreciably changed. In view of these observations, lipoid partition determinations of our fixed specimens were considered inadvisable. We have found that specimens kept in formalin at room temperature show lower fat content than specimens from the same liver kept in the refrigerator, probably because of changes in the properties of certain phospholipids which either tend to diffuse into the formaldehyde or, upon treatment with formaldehyde, become less soluble in fat solvents such as chloroform. It must be emphasized that the analyses made on formalin-fixed liver tissues do not represent accurately the concentrations of fat in the fresh tissues but must be considered merely as reflections of the fresh tissue values. Each fat analysis was done in duplicate, and the differences between the duplicate determinations varied from 0 to 0.14 Gm. per 100 Gm. of tissue, averaging 0.09 Gm. per 100 Gm. of tissue.

RESULTS

The results of the analyses of the livers of the patients in Group I are presented in Table I. The average total fat content of these livers from patients with chronic alcoholism and cirrhosis who had received amino acids was 2.16 Gm. per 100 Gm. of tissue, and the average total liver protein (calculated from the total nitrogen) was 19.0 Gm. per 100 Gm. The liver weight averaged 1,607 Gm.

In Group II, consisting of patients who had had chronic alcoholism and cirrhosis of the liver but had not received amino acids, the total fat content of the liver specimens averaged 22.06 Gm. per 100 Gm. of tissue and the total protein content averaged 13.1 Gm. per 100 Gm. The range for total fat was

TABLE I

PATIENTS WITH CIRRHOSIS OF THE LIVER AND CHRONIC ALCOHOLISM TREATED WITH AMINO ACIDS*

PATIENT	LIVER WEIGHT (GM.)	TOTAL LIVER LIPIDS GM. PER 100 GM. OF TISSUE	TOTAL LIVER NITROGEN GM. PER 100 GM. OF TISSUE	TOTAL LIVER PROTEIN GM. PER 100 GM. OF TISSUE
J. R.	1,400	2.13	2.95	18.4
A. B.	2,360	1.89	3.09	19.3
E. C.	1,060	2.45	3.09	19.3
Average	1,607	2.16	3.04	19.0

*The liver weight was determined at the time of autopsy. The liver lipids and nitrogen were determined on formalin-fixed specimens. The total protein was derived from the total nitrogen determination.

from 2.23 to 35.60 Gm. per 100 Gm. and for total protein, from 3.2 to 19.0 Gm. per 100 Gm. The average liver weight was 2,738 Gm. The individual analyses are presented in Table II. [Ralli and her collaborators,[5, 6] analyzing fresh liver specimens, found that the average total lipid content of livers from twenty-five patients with a history of chronic alcoholism (including eight patients with evidences of early or advanced cirrhosis) was 12.2 Gm. per 100 Gm. of tissue, with a range of from 2.0 to 34.8 Gm. per 100 Gm. The average liver weight of their group was 2,020 Gm. The eight patients in her group with cirrhosis of various stages and alcoholic histories had an average total liver lipid of 13.0 Gm. per 100 Gm. (with a range of 2.00 to 34.8 Gm. per 100 Gm.), and an average liver weight of 2,519 Gm.]

TABLE II

PATIENTS WITH CIRRHOSIS OF THE LIVER AND CHRONIC ALCOHOLISM WHO DID NOT RECEIVE AMINO ACIDS

PATIENT	LIVER WEIGHT (GM.)	TOTAL LIVER LIPIDS GM. PER 100 GM. OF TISSUE	TOTAL LIVER NITROGEN GM. PER 100 GM. OF TISSUE	TOTAL LIVER PROTEIN GM. PER 100 GM. OF TISSUE
J. L.	1,450	2.23	3.04	19.0
G. C. B.	3,950	35.60	0.485	3.2
W. L.	3,900	32.98	1.96	12.2
C. E. D.	1,650	17.41	2.90	18.1
Average	2,738	22.06	2.096	13.1

TABLE III

PATIENTS WITHOUT PRIMARY DISEASE OF THE LIVER

PATIENT	DIAGNOSIS	LIVER WEIGHT (GM.)	TOTAL LIVER LIPIDS GM. PER 100 GM. OF TISSUE	TOTAL LIVER NITROGEN GM. PER 100 GM. OF TISSUE	TOTAL LIVER PROTEIN GM. PER 100 GM. OF TISSUE
E. I.	Hypertensive heart disease	2,500	4.33	2.52	15.7
F. A. P.	Prostatic hypertrophy and pyelitis	2,050	1.97		
A. G. D.	Hypertensive heart disease, pulmonary infarction	2,000	1.70		
F. B.	Aortic stenosis	1,700	3.68		
R. D.	Nephrosclerosis	2,000	3.11		
J. L. B.	Pulmonary tuberculosis	1,000	2.07	3.22	20.1
C. C.	Nephrosclerosis	2,100	3.19		
C. C.	Lung abscess	1,300	2.34		
W. V.	Carcinoma of colon	1,650	3.67		
T.	Myocardial infarction	2,250	4.03	3.34	20.8
Average		1,855	3.01	3.03	18.8

In Group III, consisting of patients dying of causes not related to primary disease of the liver, the total fat content of the liver specimens averaged 3.01 Gm. per 100 Gm., with a range of 1.70 to 4.33 Gm. per 100 Gm. The total protein (determined in only three instances) averaged 18.8 Gm. per 100 Gm. The liver weight averaged 1,855 Gm. [Ralli and co-workers[5] found that fresh livers from normal individuals who died of accidental injuries averaged a total lipid content of 4.98 Gm. per 100 Gm. of liver (with a range of 2.42 to 8.41 Gm.) and the liver weight averaged, 1,430 Gm.] The rather high average

liver weight of our patients in this group is due to the fact that in several patients chronic passive congestion of the liver secondary to circulatory insufficiency was present. The individual analyses are presented in Table III.

Examination of slides prepared from the livers analyzed revealed a rather close correlation between the extent of fatty infiltration evident microscopically and the amount of fat determined by analysis.

DISCUSSION

Admittedly, the number of cases is small and of little statistical significance. However, available data present very few studies of this character. Our comments here will concern themselves wholly with the type of cirrhosis described by Connor as "fatty cirrhosis."[7] The patients in Groups I and II are examples of this type of cirrhosis, as distinguished from toxic, biliary, syphilitic, and pigment cirrhosis.

From the data presented, two inferences are permissible:

(1) The percentage of liver protein in general varies inversely with the percentage of liver fat. Any discrepancies may be accounted for by changes in the glycogen content. It must be remembered that at least a part of the apparent increase in hepatic protein may be relative rather than actual, since the decrease in fat content would of itself result in an increased percentage of protein. The protective action of protein stores in the liver and body against hepatotoxic agents has been demonstrated in the experimental animal by Miller and Whipple,[8] and our analyses are suggestive confirmation of the same mechanism in man.

(2) The administration of the amino acid mixture to patients with cirrhosis of the liver and chronic alcoholism apparently resulted in a decrease in percentage of hepatic fat and a concomitant increase in percentage of hepatic protein. Of course, the ideal method of proof that the changes in liver fat and protein are a direct result of the action of the amino acids would be the determination of fat and protein content of liver biopsy specimens before and after the administration of amino acids; however, this procedure was not feasible in the patients we studied. The fact that all the patients in Groups I and II were chronically addicted to alcohol renders them a fairly homogeneous group and reduces the number of variable factors, as also does the determination of average values. It will be seen that, in Group II, the liver of only one patient (J. L.) exhibited fat and protein percentages and liver weight within the control range and within the range of specimens from patients treated with amino acids. Connor[7] states that in patients who have used alcohol excessively over a long period of time but have maintained a fairly good diet, fat may or may not be present and the liver is likely to be reduced in size.

The significance of fatty infiltration of the liver as a precursor of cirrhosis has been stressed recently.[7, 9] In experimental animals, fatty livers have been produced by high fat diets, cystine, starvation, cholesterol, and toxic agents such as alcohol, phlorizin, chloroform, carbon tetrachloride, phenylhydrazine, and phosphorus.[10, 11] In clinical medicine, fatty livers may develop in chronic alcoholism, diabetes, mellitus, pernicious anemia, poisonings with the chemicals mentioned above, yellow fever, eclampsia, acute yellow atrophy, marked obesity,

certain endocrine disorders, and lesions of the pituitary or midbrain. Fatty infiltration of the liver may lead to interference with tissue metabolism, with resultant degeneration of liver cells and replacement by perilobular fibrous tissue. It is readily seen that any agent which would decrease the rate of deposition of liver fat and accelerate its removal (i.e., a lipotropic agent[12]) might prevent the development of cirrhosis in fatty livers and would be of vast clinical significance. Recent theories of the pathogenesis of hepatic cirrhosis have been reviewed by Jolliffe and Jellinek,[13] and they express the belief that the investigations of Connor and his associates in the relationship between fatty liver and cirrhosis are among the most important advances.

The experimental background of lipotropic activity of raw pancreas, lecithin, choline, proteins, methionine, betaine, and constituents of vitamin B complex has recently been reviewed.[12] It has been shown that choline, methionine, and betaine are similar physiologically in their lipotropic activity and are similar chemically in that all possess labile methyl groups.[14] The lability of the methyl group and its potentiality for participating in transmethylation reactions may be the determining factor in the lipotropic activity of these substances and may be important in detoxification processes in the body. Lowry and co-workers[15] reported that the administration of choline or a high casein diet to rats with experimental dietary cirrhosis resulted in regeneration of liver cells, disappearance of degenerative fatty changes, and decrease in liver size. Daft, Sebrell, and Lillie[16] demonstrated that cirrhosis of the liver could be *prevented* by the administration of methionine or choline or casein, singly or in combination, to rats receiving low protein rations and alcohol. The methionine content of casein is 3 per cent,[17] and it has been shown that methionine may supply methyl groups, for the formation of choline or an active compound derived from choline,[18] and that the administration of methionine accelerates the rate of phospholipid turnover in the liver.[19] Recognizing that results in experimental animals are not directly applicable to the interpretation of metabolic changes in human beings, we believe, nevertheless, that the lipotropic activity of the amino acids administered in our study probably was due to the methionine content of the amino acid mixture, which was prepared by hydrolyzing casein, and contains amino acids in the same proportion as they are found in casein. The lipotropic activity of choline has been demonstrated conclusively,[11, 12, 15, 20] and the good results reported by Broun and Muether,[21] in treating patients with hepatic cirrhosis with choline and a low fat diet, may, in part, be due to the lipotropism of choline.

Good results have been reported in treating cirrhosis of the liver with a high carbohydrate, high protein, low fat diet, with vitamin supplements,[22, 23] and it remains to be seen whether the prognosis can be influenced *still* more favorably by the additional use of choline, methionine, or amino acid mixtures.

SUMMARY

1. Liver specimens (preserved in formalin) from patients with cirrhosis of the liver and chronic alcoholism were analyzed for their total lipid and total nitrogen content. It was found that specimens from patients who had received amino acids as part of their therapy contained a greater percentage of protein

and a lesser percentage of fat than specimens from patients who had not received amino acids.

2. It is postulated that these results indicate a lipotropic activity of the amino acid mixture, probably due primarily to their methionine content.

3. The percentages of liver fat and of liver protein were found to vary inversely.

4. The clinical significance of these findings is briefly discussed.

REFERENCES

1. Fagin, I. D., and Zinn, F. T.: Cirrhosis of the Liver: Results of Treatment with Parenterally Administered Amino Acids, J. LAB. & CLIN. MED. **27**: 1400, 1942.
2. Channon, H. J., Platt, A. P., and Smith, A. B.: The Dietary Prevention of Fatty Livers, Biochem. J. **31**: 1736, 1937.
3. Halliday, N.: The Effect of Formalin Fixation on Liver Lipids, J. Biol. Chem. **129**: 65, 1939.
4. Weil, A.: The Influence of Formalin Fixation on the Lipoids of the Central Nervous System, J. Biol. Chem. **83**: 601, 1929.
5. Ralli, E. P., Rubin, S. H., and Rinzler, S.: The Liver Lipids in Normal Human Livers and in Cases of Cirrhosis and Fatty Infiltration of the Liver, J. Clin. Investigation, **20**: 93, 1941.
6. Ralli, E. P., Paley, K., and Rubin, S. H.: The Liver Lipids and Their Distribution in Disease, An Analysis of 60 Human Livers, J. Clin. Investigation **20**: 413, 1941.
7. Connor, C. L.: The Etiology and Pathogenesis of Alcoholic Cirrhosis of the Liver, J. A. M. A. **112**: 387, 1939.
8. Miller, L. L., and Whipple, G. H.: Chloroform Liver Injury Increases as Protein Stores Decrease, Am. J. M. Sc. **199**: 204, 1940.
9. Connor, C. L.: Fatty Infiltration of the Liver and the Development of Cirrhosis in Diabetes and Chronic Alcoholism, Am. J. Path. **14**: 347, 1938.
10. Bollman, J. L.: Experimental Studies on Hepatic Alterations (Fatty Liver), Proc. Staff Meet., Mayo Clin. **11**: 727, 1936.
11. Best, C. H.: The Role of the Liver in the Metabolism of Carbohydrate and Fat. III. The Deposition of Liver Fat, Lancet **1**: 1274, 1934.
12. Frame, E. G.: Lipotropic Substances, Yale J. Biol. & Med. **14**: 229, 1942.
13. Jolliffe, N., and Jellinek, E. M.: Vitamin Deficiencies and Liver Cirrhosis in Alcoholism. Part VII. Cirrhosis of the Liver, Quart. J. Studies on Alcohol **2**: 544, 1941.
14. Editorial: Significance of the Labile Methyl Group, J. A. M. A. **119**: 1111, 1942.
15. Lowry, J. V., Daft, F. S., Sebrell, W. H., Ashburn, L. L., and Lillie, R. D.: Treatment of Dietary Liver Cirrhosis in Rats with Choline and Casein, Pub. Health Rep. **56**: 2216, 1941.
16. Daft, F. S., Sebrell, W. H., and Lillie, R. D.: Production and Apparent Prevention of a Dietary Liver Cirrhosis in Rats, Proc. Soc. Exper. Biol. & Med. **48**: 228, 1941.
17. Block, R. J., and Bolling, D.: The Determination of the Amino Acids, Minneapolis, 1940, Burgess Publishing Co.
18. Channon, H. J., Manifold, M. C., and Platt, A. P.: The Action of Sulfur-Containing Amino Acids and Proteins on Liver Fat Deposition, Biochem. J. **34**: 866, 1940.
19. Pearlman, I., Stillman, N., and Chaikoff, I. L.: Radioactive Phosphorus as an Indicator of Phospholipid Metabolism, J. Biol. Chem. **133**: 651, 1940.
20. György, P., and Goldblatt, H.: Experimental Production of Dietary Injury (Necrosis, Cirrhosis) in Rats, Proc. Soc. Exper. Biol. & Med. **46**: 492, 1941.
21. Broun, G. O., and Muether, R. O.: Treatment of Hepatic Cirrhosis with Choline Chloride and Diet Low in Fat and Cholesterol, J. A. M. A. **118**: 1403, 1942.
22. Patek, A. J., Jr., and Post, J.: Treatment of Cirrhosis of the Liver by a Nutritious Diet and Supplements Rich in Vitamin B Complex, J. Clin. Investigation **20**: 481, 1941.
23. Fleming, R. G., and Snell, A. M.: Portal Cirrhosis With Ascites: An Analysis of 200 Cases with Special Reference to Prognosis and Treatment, Am. J. Digest. Dis. **9**: 115, 1942.

LABORATORY METHODS

GENERAL

A SIMPLE METHOD FOR THE SEPARATION OF BLOOD SERUM*

LEA C. STEEVES, B.A., M.D., MONTREAL, CAN.

THE following method has been found to be simple and timesaving in the preparation of arsphenaminized serum for intraspinal therapy. The principle is adapted from commercial plasma separation and transfusion sets and can be used for several types of cell serum or cell plasma separation.

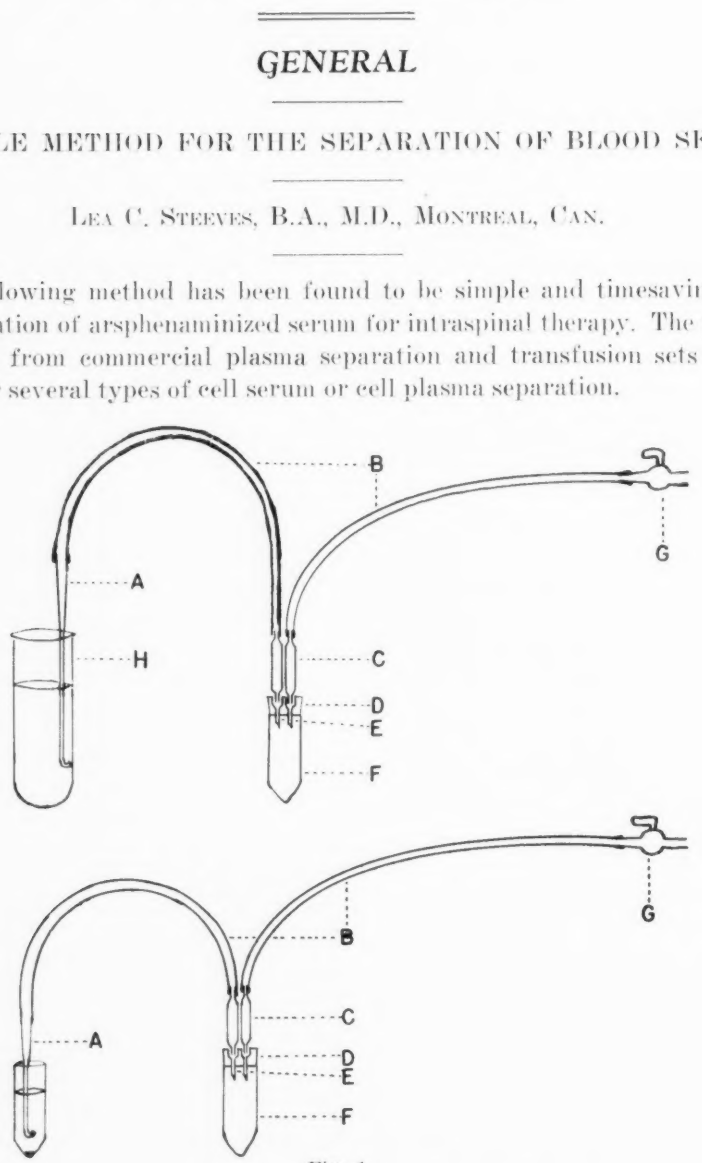


Fig. 1.

As shown in Fig. 1, two large test tubes (*H*) of about 100 c.c. capacity are provided for reception, one of initial blood, the other of final serum or plasma. Four 50 c.c. centrifuge tubes (*F*) are fitted with tight cork stoppers

*From the Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute, Montreal.

Received for publication, July 2, 1942.

Miss Doris Brophy suggested the pipettes and prepared Fig. 1.

(*D*),* through each of which two 18-gauge blood needles (*E*) are inserted. The hubs of the needles are countersunk in the cork, and one-half an inch is cut off the top of the centrifuge tube in order that the assembly may spin freely in the ordinary laboratory centrifuge. A glass adaptor (*C*), as commonly used in intravenous sets, is provided for each needle, and to each adaptor a short length of rubber tubing (*B*) is attached. A glass pipette (*A*) with approximately 18-gauge bore at the U tip is provided for each centrifuge tube. Laboratory vacuum taps (*G*) provide the necessary suction.

The entire apparatus can be neatly packed for sterilizing, wrapped under one cover, in the following units: (1) initial and final reception tubes; (2) centrifuge tubes with corks and needles inserted; (3) adaptors, tubing, and pipettes; (4) adaptors and tubing for connection to unsterile vacuum tap.

Assembly can be carried out by one ungloved operator without contamination. Serum or plasma can be separated with very little waste because of the U pipette which reduces upward currents from the cell fluid interface. By removing the adaptors, unit two can be centrifuged uncontaminated to remove residual blood cells. This procedure can be repeated as desired with loss of only a very few cubic centimeters of fluid upon each pipetting. In the case of arsphenaminized serum, Fordyce, as quoted by Moore,[1] centrifuges twice.

SUMMARY

A simple, semiclosed apparatus for rapid separation of cell serum or cell plasma preparations is described. It can be readily assembled from laboratory materials and can be used by one person unaided without danger of contamination.

REFERENCE

1. Moore, J. E.: The Modern Treatment of Syphilis, Baltimore, 1933, Charles C Thomas.

A QUADRUPLE STAINING METHOD FOR TISSUES†

S. S. KALTER, LAWRENCE, KAN.

RECENT advances in staining procedures show a definite trend toward the employment of various differential staining methods for the study of normal as well as pathologic tissues. The evolution of staining techniques need not be discussed here, as the interested student need only to turn to a monograph by Conn[1] for further information.

The foundation for this quadruple stain was Flemming's triple stain (1891), which appears to be the basis for various other multiple staining methods. It seems, however, for obvious reasons, that histologists and pathologists, in the

*Tapered rubber stoppers give a more air-tight fit but must be renewed after fifteen to twenty autoclavings.

†From the Department of Bacteriology, University of Kansas.
Received for publication, July 7, 1942.

main, restrict their tissue staining to hematoxylin-eosin, with Mallory's[2] connective tissue stain being employed when a tinctorial differentiation was wanted. The botanists, on the other hand, basing their procedures on Flemming's stain, as did Conant[3] and Johansen,[4] investigated the applicability of more complex procedures for the study of mitosis as well as differentiation of botanical tissues.

Dratman,[5] following Conant's work, proceeded to introduce this technique for the study of animal tissue. The work herein described is, therefore, a continuation of the work of Dratman. This technique is essentially that of Dratman, with modifications made to insure more constant results and to bring out sharper differentiation of the cellular elements.

METHOD

Tissues are fixed and dehydrated by any preferred method. We have given preference to Bouin's fixative, as it was demonstrated by Baird,[6] to produce a minimum amount of shrinkage and distortion in the cellular elements, with uniform results and delicacy of fixation. In using Bouin's solution, the picric acid must be removed by using a few drops of lithium carbonate in the 70 per cent alcohol dehydration series. In the use of Zenker's fixative the mercuric chloride must be removed.

The tissues, after being embedded in paraffin, give the best staining results when sectioned at 7.5 micra. In affixing the tissues to slides, the loss of tissues is negligible if the slides are thoroughly dried in an incubator at 37° C. overnight. The use of albumin water causes a cloudiness to appear in the staining reaction.

Of utmost importance is the preparation of the dyes. The dye solutions prepared as follows, have been found to give the best results:

1. Safranin O:* 0.2 Gm. of safranin O in 100 c.c. of 50 per cent ethyl alcohol plus 4 c.c. of formalin to act as a mordant and 0.5 Gm. of sodium acetate to accentuate the color.

2. Gentian violet (crystal violet):† 0.5 Gm. in 100 c.c. water.

3. Fast green FCF:‡ A saturated clove oil solution is made using 0.5 Gm. of the dye in 80 c.c. of the oil. The dye is left in the clove oil twenty-four hours with occasional shaking, then filtered through *cotton*. To 40 c.c. of this solution is added 30 c.c. of orange II.

4. Orange II:§ Saturate clove oil with 0.5 Gm. of the dye and allow to stand twenty-four hours with occasional shaking and filter through *cotton*.

The staining procedure is as follows: (1) Slides run down to tap water; (2) stained in safranin O, 24 hours; (3) tap water wash; (4) gentian violet, 1 to 2 minutes; (5) tap water wash; (6) 50 per cent ethyl alcohol, 2 minutes; (7) 95 per cent ethyl alcohol, 2 minutes; (8) fast green-orange II—differentiate under the microscope after 5 minutes; (9) pure clove oil for 10 minutes; (10) pure orange II—differentiate under the microscope after 15 minutes; (11) xylol (two changes), 10 minutes each.

*Henry Heil Chemical Co., US-8; National Aniline and Chemical Co., Certification No. NS-8.

†Gruber 9.37; National Aniline and Chemical Co., Certification No. NC-18.

‡National Aniline and Chemical Co., Certification No. NGF-4.

§Coleman and Bell Co.

In steps 8 and 10 the time expressed is a minimum due to various factors such as thickness of the tissues and cellular activity. The tissues are differentiated under the microscope in step 8 and are removed as soon as the connective tissue is green. Further differentiation takes place in the pure clove oil as well as in the orange II; therefore, clearing in the pure clove oil for ten minutes is essential.

RESULTS

Nuclei stain red, with the nucleoli staining purple or a bright purple-red, while the nuclear membrane stains a dark red. The cellular cytoplasm stains from pink to red, with a marked exception being noted in the medulla of the kidney. Here the cytoplasm of the cells making up the descending and ascending limbs of Henle's loop stain light green. The connective tissue stains green, while large elastic fibers or areas where elastic tissue predominates are sharply differentiated in yellow. The fibroblasts are green with purple nuclei. Muscular tissue usually stains a light tan, with the striations as dark bands. Red blood cells are orange. The polymorphonuclears have purple nuclei.

DISCUSSION

This method, although it is a differential connective tissue stain, cannot be compared with Mallory's. The time element as well as the striking connective tissue differentiation obtained by Mallory's stain makes it one of the most outstanding differential techniques now employed.

On the other hand, the main feature obtained by this quadruple stain is the benefits obtained by students of histology. Because of the lightness of the colors, the various elements of the tissues are not masked. The tissues can then be studied from the structural aspects as well as by the differential staining.

REFERENCES

1. Conn, H. J.: History of Staining, Geneva, N. Y., 1933, Commission on Standardization of Biological Stains.
2. Mallory, F. B.: A Contribution to Staining Methods, J. Exper. Med. **5**: 15, 1900.
3. Conant, G. H.: Conant's "Triarch" Quadruple Stain, Histological Technic, Krajian, A. A., St. Louis, 1940, The C. V. Mosby Company.
4. Johansen, D. A.: A Quadruple Stain Combination for Plant Tissue, Stain Tech. **14**: 125, 1939.
5. Dratman, M. L.: A Quadruple Stain for Animal Tissue, B.A. Thesis, LaSalle College, Philadelphia, Pa., 1940.
6. Baird, T. T.: Comparative Study of Dehydration, Stain Tech. **13**: 9, 1938.

SURFACE GROWTH OF CLOSTRIDIUM WELCHII

EDWARD STEINFIELD, M.D., AND MARGARET WATSON, PHILADELPHIA, PA.

DUE to the recent investigations of Kligler and Guggenheim,[1] it has been demonstrated that the determining factor in anaerobiosis is the reduction potential of the medium rather than other factors. In their experiments they found that growth of *Clostridium welchii* occurred in tubes with over 95 per cent of normal oxygen tension, provided an adequate amount of ascorbic acid was present. The reduction potential at which growth occurred was about –0.125 volt. These observations supplemented earlier reports of Lepper and Martin[2] who found by potentiometer and indicator methods that the Eh of freshly prepared cooked meat mediums was of the order of –0.174 volt to –0.2 volt, 1 or 2 cm. from the surface. Since cooked meat medium with a petrolatum seal is uniformly satisfactory in the cultivation of *Cl. welchii* and similar anaerobes, it is obvious that the reduction potential can be taken as a measure of the availability of an anaerobic method regardless of the hypothetical explanation of its mechanism. We have had occasion to verify the statements of Lepper and Martin and have obtained similar readings of the Eh of sealed cooked meat medium tubes by indicator methods.

The practical implication of the work of Kligler and Guggenheim is in the possibility of surface growth of *Cl. welchii* and similar anaerobes which are important, particularly in gas gangrene. Among the various methods which make this possible are the Spray anaerobic dish and the Brewer anaerobic jar. The latter is particularly valuable in the cultivation of obligate anaerobes. The possibility of using stock equipment and material has led us to improvise upon the observations of Kligler and Guggenheim with the use of the ordinary Petri dish.

The method is as follows:

Test tubes containing 12 to 15 c.c. of plain agar are heated to melting point and allowed to cool slowly as in the usual preparation for blood agar plates. Sterile defibrinated blood* is added and immediately thereafter 50 mg. of the sodium salt of ascorbic acid. The latter can be obtained commercially in the form of one cubic centimeter ampules, each containing 100 mg. of sodium ascorbate in solution. These may be diluted with equal volumes of normal saline solution and the appropriate amount added to the blood agar tube. The treated blood agar is poured into sterile Petri dishes and allowed to solidify. Within one-half to one hour the plate darkens due to the reduction of hemoglobin. Circular seals of cellophane slightly smaller than the inner compartment of the Petri dish are cut out, immersed in 70 per cent alcohol for several minutes, and

From the Laboratory of the Jewish Hospital, aided by a grant from the Sidney A. Aloe Research Fund.

Received for publication, July 7, 1942.

*Horse blood is preferable in demonstrating clear-cut beta hemolysis by *Cl. welchii*, but sheep or human blood may be used for convenience.

dried between layers of sterile filter paper. They may be kept in several such layers within sterile Petri dishes. Autoclave sterilization is less desirable since it tends to wrinkle the cellophane. We have found the product commercially labeled "No. 300 moisture proof" quite satisfactory. After streaking cultures or material presumably containing *Cl. welchii* over the surface of the treated blood agar, a cellophane seal handled with a flamed forceps is gently placed over the agar surface and rolled smoothly against it with a bit of sterile glass tubing threaded upon a wire. By this means the oxygen tension is kept to a minimum; the plate can be examined by transmitted light and handled in any position. Subcultures can easily be made by lifting the edge of the cellophane cover with forceps without undue distortion of the surface. Hemolysis can be judged, though not quite so sharply as in the usual blood agar plate because of the reduced hemoglobin. It is quite possible to obtain somewhat similar results with stored blood agar plates by applying the same amounts of sodium ascorbate to the surface of the plate and carrying the material along with the platinum loop or sterile swab during streaking. In such preparations it is possible to note the areas where the reducing action is lacking with consequent absence of growth. The method in general has so far been applied to strains growing under laboratory conditions. However, since these cultures fail to grow by ordinary aerobic methods, it can be assumed that their anaerobic properties are still maintained. In order to verify the sealing effect of the cellophane, agar plates containing the sodium ascorbate and an indicator such as 2, 6-dibromophenolindophenol were set up and kept at room temperature. All plates showed decolorization of the dye, but controls without cellophane gradually exhibited oxidation from the surface downward; those with cellophane remained decolorized for from seven to ten days. Although this indicator does not register the optimum Eh which can be obtained, it is useful in this particular observation because of its clearcut readings.

There are certain disadvantages to the method. So far it has been found difficult to apply to the sugar mediums and for the culture of *Cl. tetani*. A moisture film may make single colony isolation difficult. The expenditure for the sodium ascorbate would have to be considered in large scale observations. It may be advisable to adapt this method, if possible, to the use of such reducing mediums as cysteine hydrochloride or sodium thioglycollate. These have recently been reviewed and amplified by Brewer.[3] Spray[4] has also mentioned the use of flat sheets of cellulose over deep agar plates. Although these approaches to surface culture are no doubt being investigated, we feel that this brief description may induce trial and improvement of such methods. A similar technique may prove useful in some exigencies of time and material or where the use of hydrogen jars is undesirable.

REFERENCES

1. Kligler, I. J., and Guggenheim, K.: Influence of Vitamin C on Growth of Anaerobes in the Presence of Air, J. Bact. **35**: 141, 1938.
2. Lepper, E., and Martin, C. J.: Oxidation-Reduction Potential of Cooked Meat Media, Brit. J. Exper. Path. **11**: 137, 1930.
3. Brewer, J. H.: Clear Liquid Mediums for the "Aerobic" Cultivation of Anaerobes, J. A. M. A. **115**: 598, 1940.
4. Spray, R. S.: Composition Tubes as Substitute for Glass in Isolation of Sporulating Anaerobes, J. Lab. & Clin. Med. **26**: 1957, 1941.

ADAPTATION OF A KAHN SHAKER FOR HOMOGENIZING SPECIMENS FOR BACTERIOLOGIC PROCEDURES

LILLIAN BUXBAUM,* M.S., NEW YORK, N. Y.

IN THE course of our laboratory work it is often necessary to shake vigorously several different types of bacterial samples to obtain homogenous suspensions. To expedite and simplify this procedure, a mechanical shaker is considered desirable. A Kahn shaker that was already part of our laboratory equipment was satisfactorily adapted for our needs. The idea for adapting a Kahn shaker was obtained from a bottle shaker manufactured by the Precision Scientific Company of Chicago (catalogue No. 5892).

Fig. 1.

Lead-coated copper, which is rust-resistant, 1 mm. in thickness, was used to make a tray 14 inches long, 12 inches wide, and 2 inches deep (Fig. 1). Specifications of the tray were limited by the apparatus already in the shaker which is used for shaking hemocytometers. Both the tray and the latter apparatus are fitted into the ledge of the shaker. The tray is removable so that it can be easily cleaned and sterilized.

Metal holders (Fig. 2) were ordered from the Standard Scientific Supply Company of New York. Spring brass was the metal of choice for these holders because of its malleability and tensile strength. Two 1 mm. metal bands, 1 inch wide and 6 inches long, are placed at right angles and soldered together. The bands are bent at a 90-degree angle so that the four upright arms are 2 inches in height. The four ends are curved in order to hold the specimen containers

Received for publication, July 14, 1942.

*Assistant Bacteriologist, Pathological Laboratory, Willard Parker Hospital.

securely. The width at the base of the finished holder is 2 inches. The top can be expanded to 4 inches or contracted to 2 inches, depending on the size of the container used. Rivets are used to attach the holders to the tray at a distance of three and one-half inches from the center of each holder.

The shaking apparatus, thus modified, was utilized at first to homogenize sputum for concentration of *Mycobacterium tuberculosis*. Half pint wax cups with a 2-inch base diameter, a 3-inch brim diameter, and 3½ inches in height were used as containers for these specimens. All tenacious masses were completely broken up, and good digestion of the sputum was obtained after adding 3 per cent sodium hydroxide and shaking for twenty minutes.

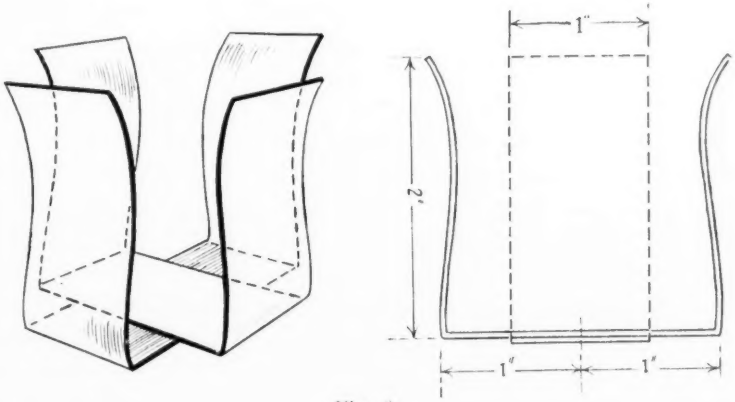


Fig. 2.

Due to the fact that the holders can be adjusted, different sizes and types of containers can be inserted and held securely by the flexible metal strips. Among other uses, it was found that homogenous suspensions of streptococcus growth in some liquid media resulted after shaking for from four to six minutes. As this organism produces a granular growth in broth, the shaker proved invaluable when used in preparing different amounts of antigens for the Griffiths' typing of hemolytic streptococci.

The hospital tinsmith, Frank J. Musacchia, Jr., constructed the tray and riveted the holders.

I wish to thank Dr. Vera B. Dolgopol for her suggestions which aided in the preparation of this paper.

ROLLER RIM-DRIVE KYMOGRAPHS

R. P. Walton, M.D., F. M. Cook, B.S., and A. B. Cullen, M.S.,
Charleston, S. C., and University, Miss.*

KYMOGRAPHS have always represented one of the major equipment problems of pharmacology and physiology laboratories. The chief difficulty in their construction is occasioned by the need for wide ranges of speed, and, at present, these are obtained almost exclusively by shifting gears or rearranging belts, with usually a rheostat or governor control of the motor. These motor controls are limited to a 6-to-1 range usually, or as a maximum, a 10-to-1 range. Additionally, the rheostat control even at the 6-to-1 range involves serious loss of torque at the lower speeds, since its effect is essentially to dissipate power. This, similarly, is a disadvantage of fan-controlled spring motors.

A distinct improvement will be the development of some device which will give smooth, continuous control of small motors over a speed range of about 100 to 1. By the use of reducing gears, two such speed areas could be made available with only one shift of gears (or change of drives). Speed ranges of 10,000 to 1 would then be available, and this covers practically all the needs of pharmacologic and physiologic work. A wide range of control is now commercially available with large motors. For instance, the Crocker-Wheeler polyspeed motors operate with a variable speed 3 phase A. C. motor which has separately excited rotor and stator coils and has essentially the same characteristics as a D. C. shunt motor; that is, constant torque at different speeds.[1] Another possible type of motor control is that described by Ryder in which a D. C. motor is shunt wound and has a separately excited armature and field.[2] The armature voltage might be varied from zero to maximum by means of a grid-controlled rectifier acting as a variable voltage source, a function which can be satisfactorily performed by a thyratron tube. There are other arrangements of a purely mechanical nature by which the desired motor control might be obtained. One is by the use of small hydraulic, piston-drive motors and pumps (in which variations of fluid flow are subject to valve control). Another is by the use of a planetary drive system in which the differential rate of two motors might be subject to a wider range of control than is the case with a single motor.

We have made tentative experiments with each of these possible arrangements and in each case have found enough complications and disadvantages to preclude the immediate prospect of using them as kymograph drives which could be developed as a practical improvement over existing methods. These possibilities are mentioned, however, since with subsequent technical developments or in other hands they might be satisfactorily adapted to this particular purpose.

The arrangement which we have developed and put into use involves a simple mechanical principle which without any new meshing of gears gives the needed wide range of speeds and is capable of construction at a relatively low

*From the Departments of Pharmacology, Medical College of the State of South Carolina and School of Medicine, University of Mississippi.
Received for publication, July 17, 1942.

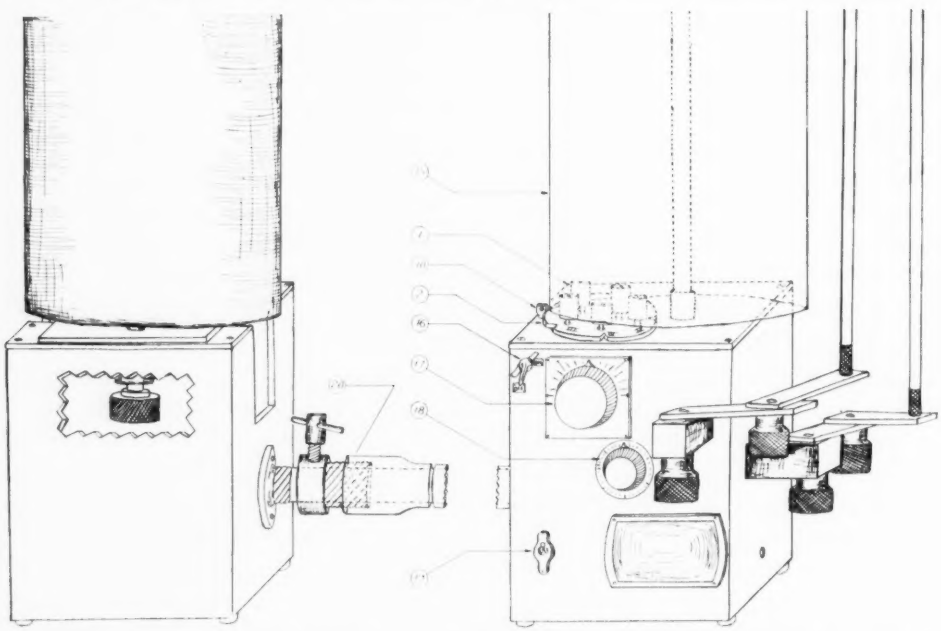
cost under ordinary shop conditions. Its chief point of novelty lies in the use of the roller rim-drive principle such as has been used in musical record players for the last several years. This is essentially a small, power-driven roller operating by friction against the inner shell of a kymograph drum. Commercial friction drives at a more central point of power have not been satisfactory in our experience, but this type of friction drive at the most peripheral point of the system has proved exceptionally satisfactory. One such drive has been used now for five years in these laboratories and has always given smooth operation without slipping. Our presently preferred kymograph design operates at top speed with a pull of ten pounds at the periphery of an eight-inch drum, and, under test conditions up to this maximum pull, there has been no slipping. This is an extreme test, since some widely used commercial kymographs are stopped by less than one-pound pull at the drum periphery.

The unique advantage of the roller rim-drive lies in the fact that several rollers can be power driven within the shell of the drum, and, by a fairly simple arrangement, any single roller can be selected for driving contact while the other rollers are allowed to idle. Thus different ranges of speed can be made immediately available without the usual changing of gears or shifting of drive belts. The fact that there is actually no new meshing of gears in any of these changes of speeds eliminates the need for the more precise type of machining construction which is necessary when several gear shifts are required.

This principle can be illustrated best by the simplest theoretical arrangement in which only two rollers are being driven inside the drum shell. These are being driven at different rates of speed by a motor and gear box in a housing below the drum. The rollers are mounted on free, movable arms of bar iron and connected by small spur gears to the power shafts. The arms rotate freely about cylinders which enclose the fixed power shafts. By two simple maneuvers, one roller can be released from contact with the inside shell of the drum and the other brought into contact. This contact can be maintained with a tension spring. With rheostat control of the motor, which usually gives satisfactory control over a range of 6 to 1, two areas of speed range are made available. This represents the simplest arrangement of multiple drives, and the construction details are simple. By increasing the number of power-driven shafts and their attached rotating arms and roller drives, additional speed ranges are made available and without the necessity of meshing new gear combinations. The mechanical difficulties of arranging side arms underneath the drum, however, increase with the number of side arms and a point may be reached where there may be interference with recording instruments.

We have found that the most suitable means of making available several such speed areas is a system which drives a number of contact rollers from one circular gear box, which itself can be freely rotated about a central power shaft coming directly from the motor. The whole gear housing can be shifted away from the drum periphery enough to release the friction contact of any of its rollers, the gear system rotated to place the selected roller drive in position for contact with the rim, and the gear housing shifted back to establish contact of the selected roller with the rim.

This gear box mechanism is of a clock-work type, and we have preferred a gear reduction of about 6 to 1 between each roller drive shaft. With five such roller drives and ordinary rheostat control, a continuous selection of speeds is available over a range of about 7,500 to 1. This permits a top speed which completely revolves a ten-foot paper in about one minute and a lowest speed which revolves the paper once in about five days. Stated differently, the paper can be driven at about 5.0 cm. per second at top speed and at about 2.4 cm. per hour at the lowest speed. Any speed within this range can be selected immediately and put in operation without disturbing the continuity of the tracing. There is no disturbing jar of the kymograph with speed changes, there is no waiting interval for a train of gears to catch up, and there is no forward jerk of the drum as new gears are meshed. These speed changes are obtained by knob controls placed out of the area of the support rods and recording instruments.



Figs. 1, 4, and 5.—*1*, Drive roller; *2*, indicator plate; *3*, gear housing; *4*, bevel gears; *5*, support for shaft to bevel gear; *6*, coupling; *7*, flexible shafting; *8*, motor mounting; *9*, drum shaft; *10*, stabilizing post; *11*, cam acting on gear housing to disengage roller; *12*, spring acting in gear housing to maintain friction of roller; *13*, rheostat; *14*, motor; *15*, drum; *16*, knob to cam shaft; *17*, knob to bevel gear; *18*, knob to rheostat; *19*, on-off power switch; *20*, spreader arrangement.

Fig. 1.—Schematic view of entire kymograph.

The details of this construction are given below. Additionally some features of kymograph assembly are mentioned which may be helpful to those particularly interested in cutting down costs. A rugged kymograph giving the above speed range and operating with adjustable large double drums can be assembled from less than $35 worth of parts in a shop equipped with a lathe and ordinary metal working equipment.

Drums.—The drums are made of laminated bakelite tubing (Synthane) which can be obtained in varying diameters and can be cut to order in various lengths. We have used such drums for five years and consider them preferable to metal drums. Heads are machined from a plastic slab or, adequately enough,

from plywood with a top covering of smooth asbestos composition board.* The lower head is fixed in the drum about one to two inches from the lower edge. This allows room for the rubber rollers to project up inside the shell of the drum. The drum itself with a central shaft of metal tubing idles on a shaft of three-fourth-inch diameter rodding attached to the base.

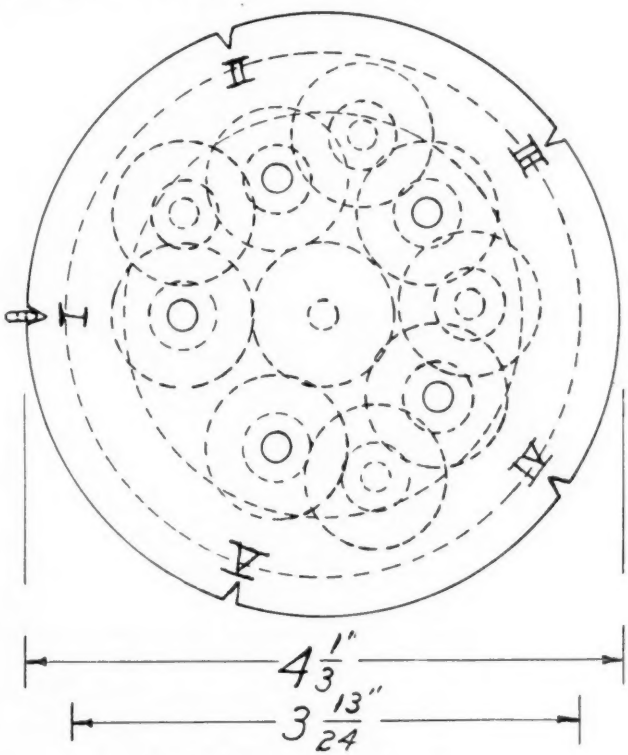


Fig. 2.—Top view of gear mechanism.

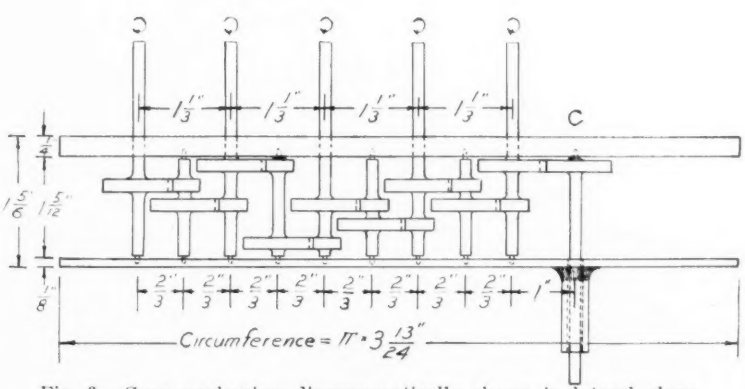


Fig. 3.—Gear mechanism diagrammatically shown in lateral plane.

Base.—The iron base is cast to order as a rectangular box weighing about thirty-five pounds. The bottom is left open and has rubber bumpers screwed up into the rim. The top is covered with a rolled steel plate.

*Decorative Flexboard, Johns-Manville Corp.

Idler Drum and Base.—The idler drum and base are the same as the motor-driven unit, except that the shaft of three-fourth-inch rodding is mounted on a movable plate, which can be slipped back and forth on the base. A heavy, knurled knob connects with this movable plate through a center groove in the top of the base. The drum bases are separated from each other by telescoping pieces of pipe with a threaded attachment for precision adjustments.

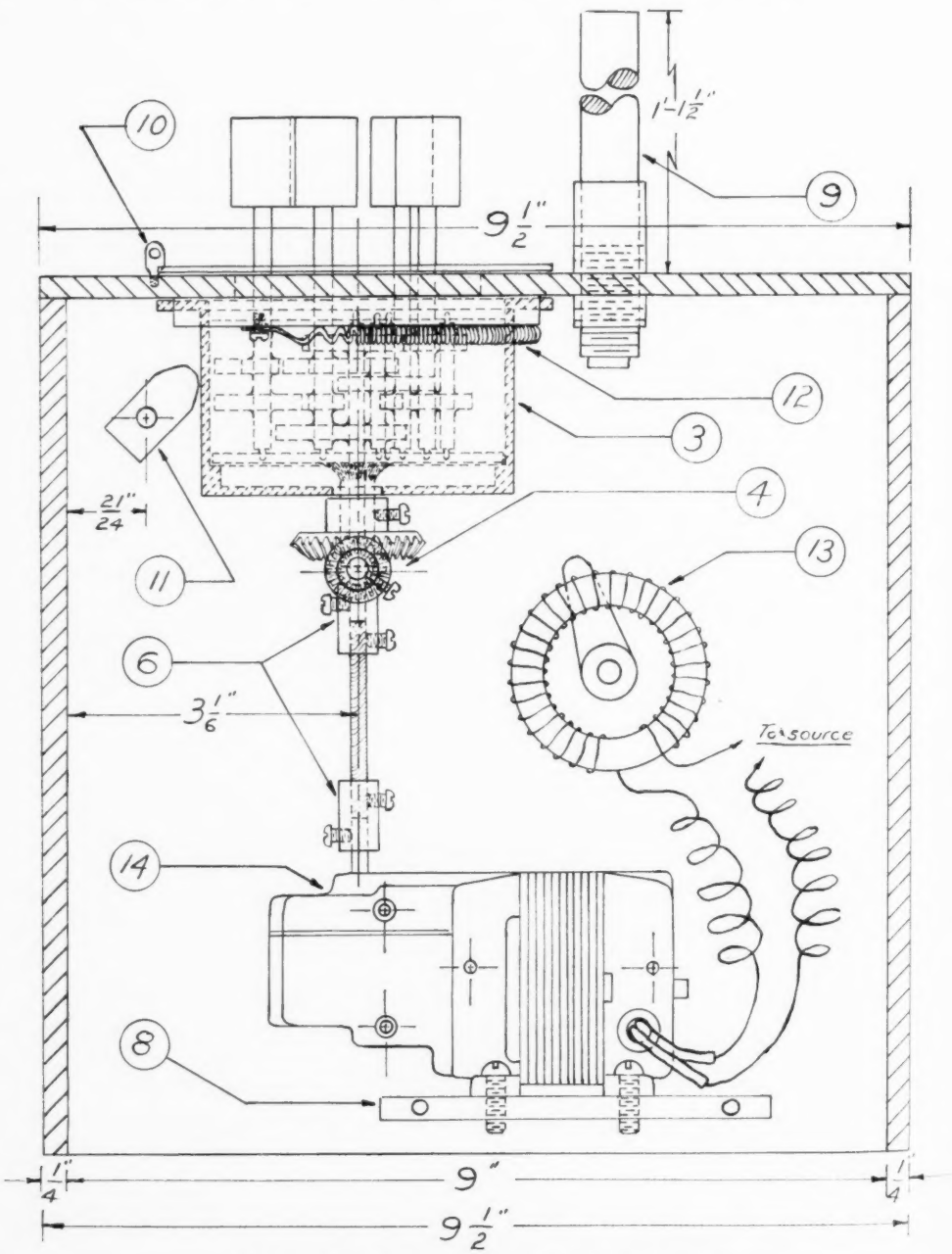


Fig. 4.—Front view (exposed) of gear mechanism.

Gear Box.—The clockwork mechanism is mounted inside a piece of four-inch pipe, and this housing is flanged at the top so as to slide in grooved backets attached to the top plate. A spring maintains tension of the entire housing toward the periphery of the drum. This gear housing is moved back away from the drum periphery by means of a cam shaft operated by a knob in the front of the base. The mechanism is made up of gears with twelve and thirty teeth each. Since each roller drive shaft must be turning in the same direction, two pairs of

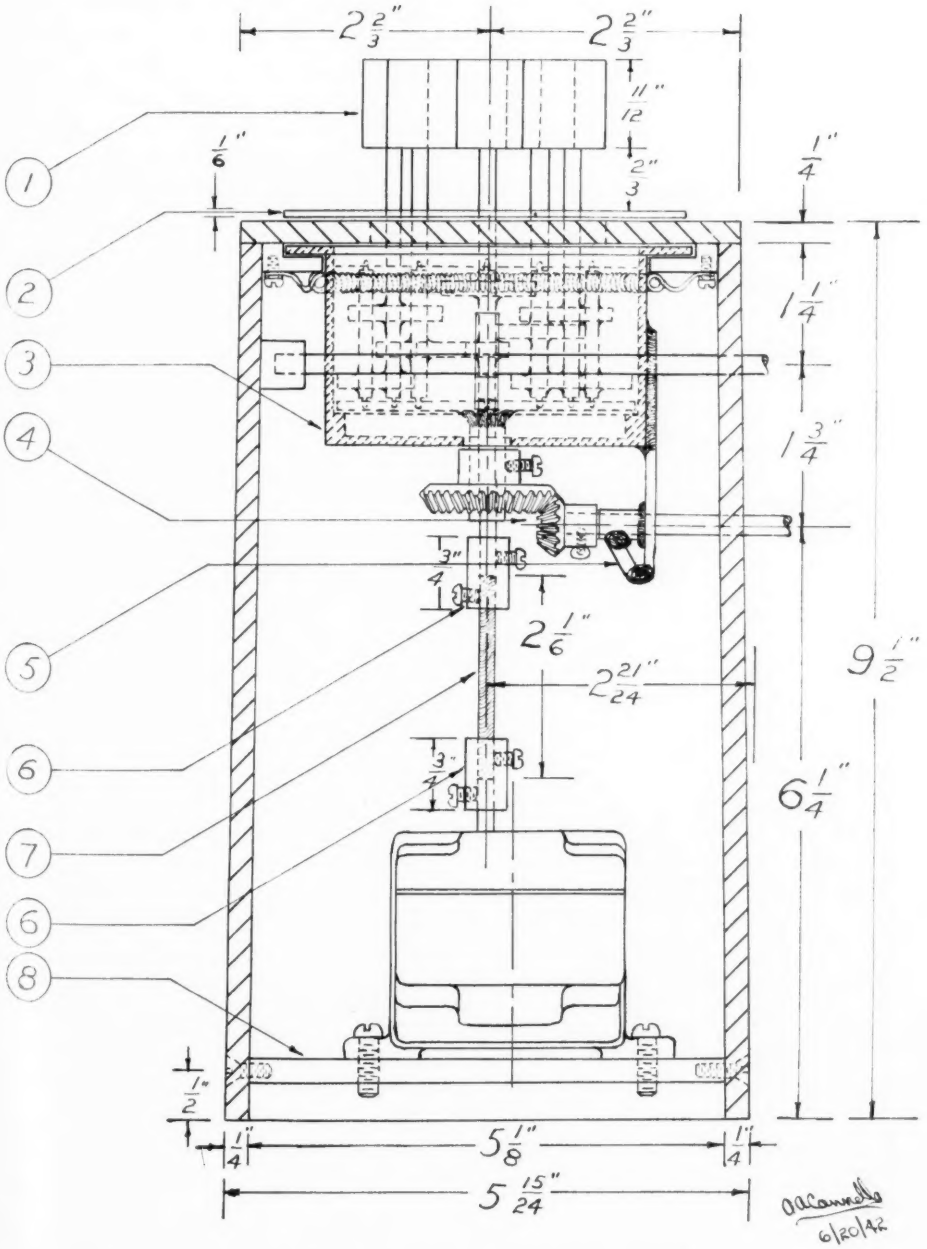


Fig. 5.—Left side view (exposed) of gear mechanism.

such gears are necessary, which, with the gear ratios of 12 and 30, make a speed reduction of 6.25 from one drive shaft to the next. The main drive shaft from the motor enters at the center of the gear housing and drives at a rate of 40 or 80 r.p.m. This low speed subjects the gear mechanism to very little wear and obviates the need for an oil bath or special oiling arrangements. This motor drive shaft is made up partly of a section of flexible shafting since the motor is stationary while the gear box is moved back and forth about one-fourth inch. The gears are mounted on a circular plate which turns smoothly on an offset within the housing and is connected by a threaded and soldered metal tube with a bevel gear placed around the central gear shaft below the gear housing. This bevel gear is turned by a smaller bevel gear which is on a shaft operated by a knob from the front of the base. This bevel gear arrangement is not entirely necessary since there is actually very little interference or inconvenience if the gear platform is simply turned by hand, the rollers and their shafts above the base plate being easily accessible. The correct spacing of the gear centers is necessary in order to minimize "back-lash," which can produce an irregular rate with an unloaded fast drum. The measurements shown in the diagram are approximate and, in setting up the gears, must be calculated more accurately from the known pitch diameter of the gears.

The drive rollers are made of ordinary rubber pressure tubing spiked on to each of the five drive shafts. Considerable variation in size of rollers is possible, which correspondingly varies the drum speeds.

LIST OF MATERIALS AND MANUFACTURERS (EFFECTIVE BEFORE PRESENT EMERGENCY)

Drums. Natural Synthane xx free-machining tubing. 8 inch inside diameter by $\frac{1}{16}$ inch wall. Two 12 inch lengths.
Synthane Corp., Oaks, Pa.

Motor with worm drive reduction.
Speedway Mfg. Co., 1834 South 52nd Ave., Cicero, Ill.

Brass gears. Spur gears. 10 with 12 teeth (No. G163)
10 with 30 teeth (No. G174)
Bevel gears. 1 pair with 18 and 36 teeth (No. G485)
Cat. No. 52. Boston Gear Works, N. Quincy, Mass.

Bases. Two iron castings of 33 lb. at .07
Memphis Machine Works, Memphis, Tenn.

Power rheostat. 75 watt, 300 ohm. No. 27-457

Flexible shafting	No. 73-290
On-off switch	No. 34-520
Bar knob	No. 55076
Fluted knobs	No. 55087

Switch plates, dial plates, power cord, crystallizing lacquer, etc.
Cat. No. 107. Allied Radio, 833 W. Jackson Blvd., Chicago, Ill.

Cold rolled steel shafting, mild steel rolled plates, bar iron, spreader pipes, etc.
Lewis Supply Co., Memphis, Tenn.

REFERENCES

1. A. C. Polyphase motors, Product Engineering, March, 1938, p. 123.
2. Ryder: Electronic control circuits for D. C. motors. Electronics, Dec., 1938, p. 20. See also "Variable Speed Through Electronic Control." Product Engineering, June, 1940, p. 244.

INTRAVENOUS ADMINISTRATION OF OXYGEN

F. S. Grodins, M.S., M.D., A. C. Ivy, M.D., Ph.D., and H. F. Adler, Ph.D.
Chicago, Ill.

A RECENT report[1] claims markedly beneficial effects from the intravenous administration of relatively minute amounts of oxygen. It was stated that therapeutic results were obtained with as little as from 0.2 to 0.3 c.c. per kilogram per minute and that this method of administration was more effective than oxygen by any other route. It seemed rather difficult to account for these results, since the amount of oxygen administered is less than 10 per cent (1 to 7 per cent) of the average basal requirement. Moreover, it has previously been reported[2, 3] that intravenous oxygen in larger amounts not only is unable to correct an existing anoxemia but may actually produce an anoxemia when none is present. In view of these facts, the problem has been reinvestigated.

METHODS

Dogs anesthetized with pentobarbital were used in all experiments. Oxygen for intravenous administration was obtained from an ordinary Linde tank fitted with a reducing valve to control the delivery pressure and a needle valve to control the flow. The gas was led through an orifice meter calibrated to measure the rate of flow in cubic centimeters per minute and was administered to the animal through two 27-gauge needles, one in each brachial vein. When oxygen was given by trachea, a closed system with suitable valves to prevent rebreathing was employed. Carotid blood pressure and respiratory movements were recorded on a smoked drum. Blood arterial oxygen was determined by the manometric method of Van Slyke.[4]

RESULTS

Effect on Respiration.—Twelve animals were studied (Table I). In ten animals with intact vagi, intravenous oxygen in amounts as small as 0.21 to 0.64 c.c. per kilogram per minute caused changes in respiration. The rate was most consistently affected, being increased in all cases. The effect on depth was more variable, increases, decreases, or no change occurring with approximately equal frequency. However, the most typical response, at least initially, appeared to be rapid, shallow breathing (Fig. 1). In larger amounts, the respiratory effects were more marked. In three vagotomized dogs, intravenous oxygen produced primarily an increase in depth, the increase in rate being less conspicuous (Fig. 2).

Effect on Blood Pressure.—The blood pressure was affected less consistently than the respiration. In general, small amounts of oxygen produced little

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.
Received for publication, July 20, 1942.

change, whereas larger amounts lowered the pressure. However, there was marked individual variation.

Cardiac Froth.—Small amounts of oxygen (0.20 to 0.35 c.c. per kilogram per minute) did not produce audible cardiac froth. Larger amounts (1.0 to 2.5 c.c. per kilogram per minute) produced audible froth in all cases. This dis-

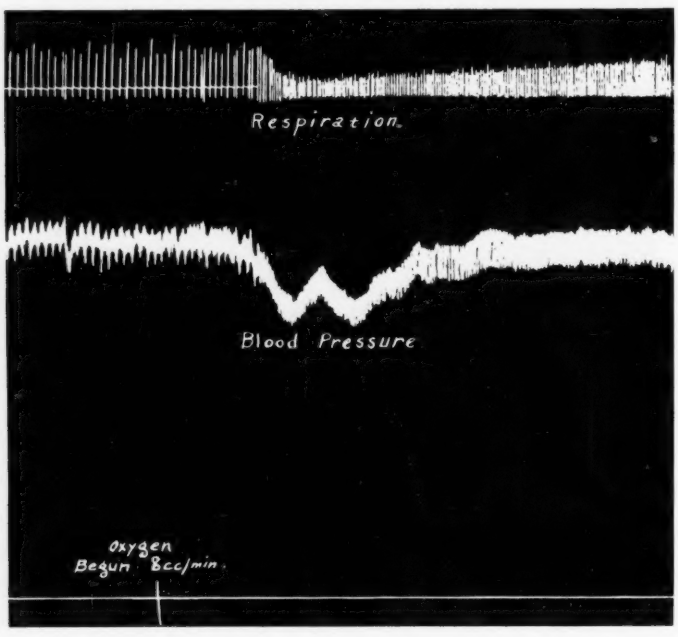


Fig. 1 (Dog 7).—Weight, 12.6 kg. Vagi intact. Intravenous oxygen at 8 c.c. per min. (0.64 c.c. per kilogram per minute). Note typical rapid shallow breathing.

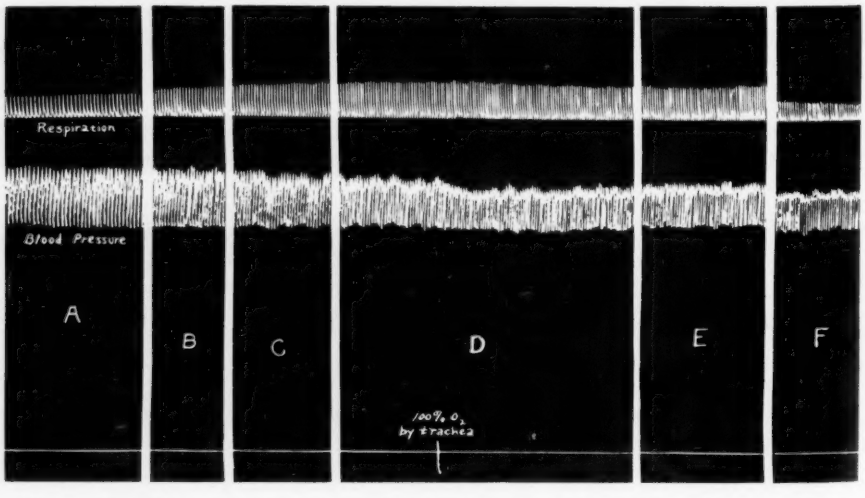


Fig. 2 (Dog 11).—Weight, 13.6 kg. Vagi sectioned. *A*, Control Tracing; *B*, intravenous oxygen 6 c.c. per minute; 0.44 c.c. per kilogram per minute; *C*, intravenous oxygen 10 c.c. per minute; 0.73 c.c. per kilogram per minute; *D*, intravenous oxygen 17 c.c. per minute; 1.2 c.c. per kilogram per minute; 100 per cent oxygen by trachea started at signal; *E*, tracheal oxygen stopped; *F*, intravenous oxygen stopped.

TABLE I

DOG	WEIGHT (KG.)	OXYGEN (C.C. PER MIN.)	OXYGEN (C.C. PER KG. PER MIN.)	RESPIRATION		BLOOD PRESSURE		ARTERIAL O_2 CONTENT		
				RATE	DEPTH	CONTROL	O_2	CONTROL	O_2	PER CENT CHANGE
1	18.2	5	0.27	++	++	128	128			
2	15.9	5	0.31	+++	+++	128	128			
3	13.6	5	0.37	++	++	166	124			
		15	1.10	++	++++	170	114			
4	11.8	2.5	0.21	+++	−−	114	86			
		10	0.85	++	−−	126	116			
		14	1.18	++	−−	120	110			
5	9.0	2	0.22	+++	−−	154	118			
		15	1.67	++++	∓	160	94	14.7	9.7	−34
6	13.6	3	0.22	+++	0	142	142	19.4	20.0	0
		15	1.10	+++	0	140	140	19.4	19.4	0
7	12.6	8	0.64	+++	−−	180	142	10.8	8.0	−18.5
		40	3.17	−−	−−	170	0			
8	10	3.5	0.35	+++	−−	132	102	10.8	7.8	−27.8
9	13	6.5	0.50	+++	0	166	156	21.6	19.7	− 8.8
		30	2.3	+++	0	156	140	21.6	20.2	− 6.5
100% O_2 by trachea		30	2.3	+++	0	140	160	21.6	23.5	+ 8.8
Vagi cut		6.5	0.50	0	+	164	168			
		30	2.3	+	++	168	154			
10	18	5.5	0.35	++	−−	154	134	20.1	17.8	−16.4
11	13.6 (vagotomized)	6 to 10	0.44 to 0.73	+	++	190	190			
		6 to 17	0.44 to 1.2	+	++	190	190			
100% O_2 by trachea		17	1.2	0	0	190	190			
12	18 (vagotomized)	6 to 16	0.33 to 0.89	+	+	160	142			
100% O_2 by trachea		16	0.89	-	-	140	120			

appeared quite rapidly (five to ten minutes) after the intravenous oxygen was stopped.

Arterial Oxygen Content.—Blood arterial oxygen was followed in six animals. In five of the six cases, intravenous oxygen in amounts varying from 0.35 to 2.3 c.c. per kilogram per minute decreased the arterial oxygen in amounts varying from 6.5 to 35 per cent.

Fatal Dose.—Dog 7 was killed by 3.2 c.c. per kilogram per minute intravenously.

DISCUSSION

In general, our results are in agreement with those of earlier investigators[2, 3] in so far as changes in respiration, blood pressure, and arterial oxygen are concerned. However, we found these changes to occur with smaller amounts of oxygen than were employed by these workers. From a physiologic viewpoint, several questions arise.

1. *Mechanism of Respiratory Changes.*—The most typical respiratory reponse to intravenous oxygen was a rapid shallow breathing (Fig. 1). This is the type of response reported by Binger, Brow, and Branch,[5] who produced multiple pulmonary emboli by the intravenous injection of starch granules in dogs, and by Dunn,[6] who performed a similar experiment in the goat. In the latter's experiments, no anoxemia was observed. In Binger's experiments, anoxemia

was produced. However, correction of the anoxemia by the inhalation of 90 per cent oxygen did not change the respiratory response. Freezing of the vagi, however, changed the response to a slow, deep respiration. These authors attributed the rapid shallow breathing to an increased sensitivity of the Hering-Breuer reflex due to the mechanical effects of pulmonary damage.

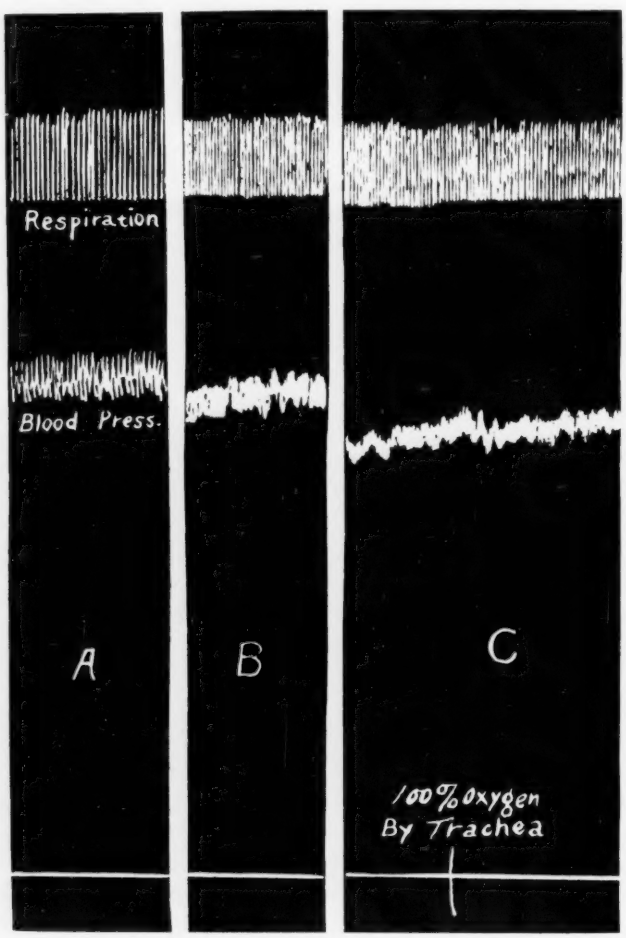


Fig. 3 (Dog 9).—Weight 13 kg. Vagi intact. *A*, Control; *B*, intravenous oxygen 6.5 c.c. per minute; 0.50 c.c. per kilogram per minute; *C*, intravenous oxygen 30 c.c. per minute; 2.3 c.c. per kilogram per minute; 100 per cent oxygen by trachea started at signal.

The respiratory response in our animals with intact vagi also was not primarily due to anoxemia, as demonstrated by Dog 6, in which rapid breathing occurred without the presence of anoxemia, and by Dog 9, in which correction of the anoxemia by the inhalation of 100 per cent oxygen failed to affect the respiratory response (Fig. 3). However, that the response is not entirely a vagal reflex phenomenon arising in the pulmonary tissue is shown by the fact that vagotomy in our animals did not abolish the response entirely, although it changed its character. In these instances, chemical factors were undoubtedly in operation.

From these considerations, the mechanism of the respiratory response to intraveous oxygen apparently depends upon two factors:

1. Pulmonary reflex via the vagi; results in rapid breathing.

2. Carbon dioxide excess and oxygen lack produced by the reduction of the pulmonary capillary bed by multiple gas emboli. According to Schmidt,[7] the typical response to these chemical stimuli is an increased depth of respiration.

In intact animals, the first factor is most important; in vagotomized animals, the second factor is dominant. For example, in Dog 11, which was vagotomized, 100 per cent oxygen by trachea only slightly decreased the respiratory response (Fig. 2). In Dog 12, however, (also vagotomized) 100 per cent tracheal oxygen markedly diminished the hyperpnea (Fig. 4). In the former case, it can be assumed that the respiratory drive was primarily centrogenic, produced by excess carbon dioxide on the respiratory center. In the second animal, which was very deeply anesthetized, the respiratory drive was chiefly reflexogenic due to the effect of anoxemia on the carotid sinus. This latter case is exactly analogous to the "oxygen inhibition" reported by Marshall and Rosenfeld[8] to occur in deeply nembutalized dogs.

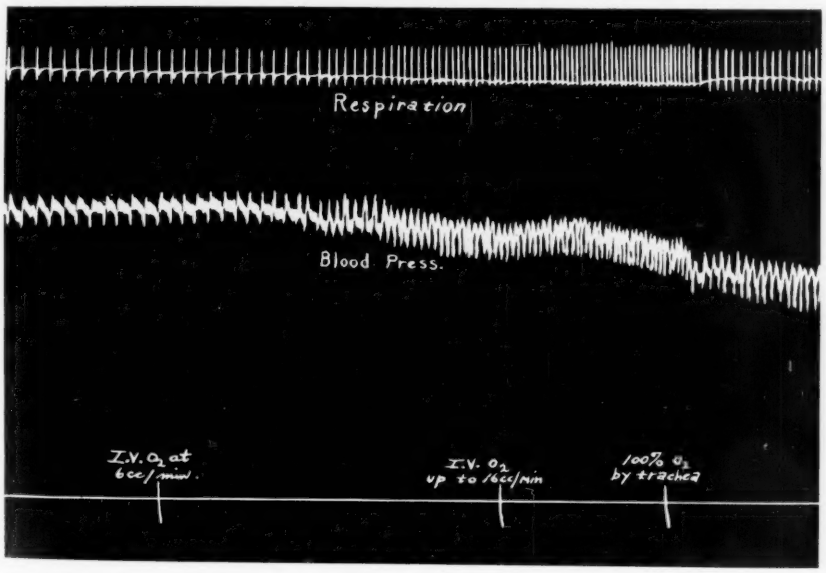


Fig. 4 (Dog 12).—Weight 18 kg. Vagi sectioned. Note inhibiting effect of 100 per cent oxygen by trachea on hyperpnea caused by intravenous oxygen.

2. *Mechanism of the Anoxemia.*—This is probably due to an anoxic anoxia caused by a reduction in the pulmonary capillary bed by multiple gas emboli. The oxygen supplied by vein cannot compensate for the reduced pulmonary supply. With larger amounts, decreased efficiency of the pulmonary circulation due to air bubbles in the right heart is undoubtedly important.

From a therapeutic viewpoint, our results fail to support the claims of Viegler.[1] Intravenous oxygen was not only unable to correct an existing anoxia produced by nembutal anesthesia, but actually increased the anoxemia. That nembutal anoxemia can be corrected by oxygen inhalation has been reported by Schnedorf[9] and McClure and associates,[10] and confirmed by us in Dog 9.

COMMENT

It would appear that if intravenous oxygen therapy will ever prove to be efficient, some way will have to be found so that significant amounts can be administered without causing bubble formation.

SUMMARY

1. Intravenous oxygen in relatively small amounts causes marked respiratory changes, may cause a fall in blood pressure, and usually reduces the arterial oxygen content.

2. The mechanism of the respiratory response and the anoxemia has been discussed.

3. Our results indicate that the intravenous administration of oxygen at rates varying from 0.35 to 2.3 c.c. per kilogram per minute is not only unable to correct an existing anoxemia, but actually aggravates the condition.

REFERENCES

1. Ziegler, E. E.: Intravenous Administration of Oxygen, J. Lab. & Clin. Med. **27**: 223, 1941.
2. Bourne, G., and Smith, R. G.: The Value of Intravenous and Intraperitoneal Administration of Oxygen, Am. J. Physiol. **82**: 328, 1927.
3. Dick, M.: Respiratory and Circulatory Responses to Intravenous Oxygen and Their Relation to Anoxemia, Am. J. Physiol. **127**: 228, 1939.
4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, vol. 2, Baltimore, 1932, Williams & Wilkins Co.
5. Binger, C. A. L., Brow, G. R., and Branch, A.: Experimental Studies on Rapid Breathing. I. Tachypnea, Independent of Anoxemia, Resulting From Multiple Emboli in the Pulmonary Arterioles and Capillaries, J. Clin. Investigation **1**: 127, 1924; Experimental Studies on Rapid Breathing. II. Tachypnea, Dependent Upon Anoxemia, Resulting From Multiple Emboli in the Larger Branches of the Pulmonary Artery, J. Clin. Investigation **1**: 155, 1924.
6. Dunn, J. S.: The Effects of Multiple Embolism of the Pulmonary Arterioles, Quart. J. Med. **13**: 129, 1919.
7. Schmidt, C. F.: Macleod's Physiology in Modern Medicine, St. Louis, 1941, The C. V. Mosby Co., chap. 28.
8. Marshal, E. K., Jr., and Rosenfeld, M.: Depression of Respiration by Oxygen, J. Pharmacol. & Exper. Therap. **57**: 437, 1936.
9. Schnedorf, J. G.: Oxygen Therapy in Reactions Following Barbiturate Anesthesia and Cisternal Intervention, Surg., Gynec. and Obst. **69**: 305, 1939.
10. McClure, R. D., Hartman, F. W., Schnedorf, J. G., and Schelling, V.: Anoxia. A Source of Possible Complications in Surgical Anesthesia, Ann. Surg. **110**: 835, 1939.

AN EFFICIENT APPARATUS FOR THE PREPARATION OF PARENTERAL SOLUTIONS

ALEXANDER G. KELLER, B.Sc., PHILADELPHIA, PA.

AS THE importance of parenteral solutions has grown to such a magnitude during the last ten years, many hospitals have transferred the preparation of these solutions from the operating room staff to the laboratory department, where a special room and personnel are used for the work.

This transfer occurred in our hospital approximately eight years ago, and from one small room we recently expanded to two rooms with a total area of 500 square feet. The rooms are adjoining, one for the cleaning of the glassware and the other for the preparation and bottling of the solutions.

PART I

The cleaning of the glassware is conducted in several steps: (1) the glassware is boiled for fifteen minutes in a detergent solution in a tank; (2) it is thoroughly rinsed with hot tap water for ten minutes; and (3) it is rinsed with distilled water for three minutes (Fig. 1).

For a tank in which to heat the glassware, we use an enameled bathtub. The tub is filled to the overflow level, capacity fifty gallons, with tap water. A wetting agent* is added to remove the hardness of the water and to prevent the formation of a precipitate when the trisodium phosphate is added. Sufficient phosphate is added to give a concentration of approximately 0.5 per cent.

After boiling the glassware the liquid is drained from the tank and the glassware is transferred to the rinsing apparatus. This consists of a shallow bathtub with two pipes, lying lengthwise, on the bottom of the tub. Each pipe has eight vertical nozzles. A wooden rack rests on the pipes, holding a flask mouth down, over each nozzle. By opening a valve, hot tap water enters the two pipes and nozzles and sprays the inside of each flask. The outside of each flask is washed by a rubber tube operated manually by the technician. Finally by closing the hot tap water valve and opening an adjacent valve, distilled water is admitted through the system, and any tap water adhering to the glassware is removed.

In order to have the distilled water under sufficient pressure for the rinsing, we use a vertical style autoclave to which is attached an electric pressure pump.

When the rinsing is completed, each flask is removed and a piece of parchment paper, freshly rinsed with distilled water, is placed over the mouth.

From the Biochemical Laboratories of the Graduate Hospital, University of Pennsylvania.
Received for publication, Sept. 23, 1942.

*Prepared by the Amalgamated Chemical Co. under the trade name Litho Grains No. 2.

Fig. 1.

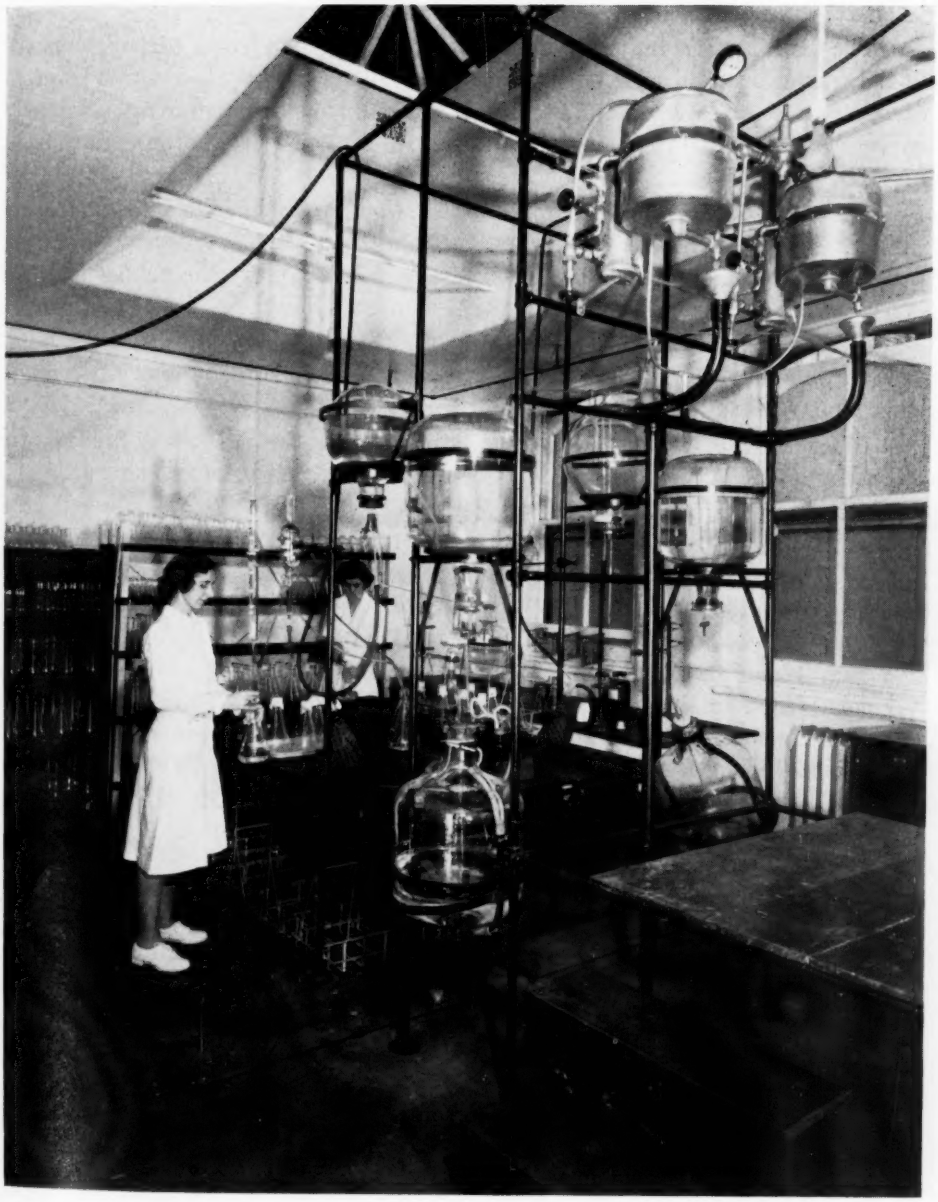


Fig. 2.

PART II

Water is distilled fresh each day by two* five-gallon-per-hour steam-heated stills,* mounted on an iron framework approximately ten feet from the floor (Fig. 2).

The distillate is collected in an inverted twelve-gallon pyrex carboy, mounted in the framework at a level below that of the stills. Directly below this inverted carboy is a second twelve-gallon carboy held in a metal harness attached by a swivel arrangement to the upright on each side. This permits the carboy to be inverted when desired.

The constituents for a parenteral solution are delivered into this lower carboy. The fresh distilled water enters from the upper carboy through a glass tube. In the center of this lower carboy, held in place by the rubber stopper, is a graduated glass tube to indicate the volume of liquid.

Mixing is accomplished by drawing air through the liquid, using a water suction pump. The air is cleaned before entering the liquid by passing through soda lime, weak sulfuric acid, and distilled water.

After mixing, the liquid is drawn by suction through glass tubing to a 50 L. spherical flask adjacent to the upper carboy. In this transit the liquid is filtered through a fritted glass pyrex filter, medium porosity. As a partial vacuum must be created, a spherical flask is used in preference to a standard-shaped bottle. The perforated rubber stopper has a metal disk attached to it on the outside of the flask to prevent the stopper from being sucked in should the vacuum become too great.

The partial vacuum is created by a water suction pump. A glass check valve is placed in the line betwen the pump and the flask to prevent backfire. A gauge is also in the line so the number of inches of vacuum can readily be seen. The vacuum should not exceed fifteen inches. As soon as the spherical flask is half full, the water suction pump is stopped and the vacuum permitted to decline voluntarily. When room pressure is reached, the liquid is permitted to enter the dispensing apparatus.

The dispensing apparatus is essentially a large burette with an overflow. It is operated with pinchcocks and rubber tubing in place of a glass stopcock.

The previously cleaned Erlenmeyer flasks are filled to half their capacity from the dispensing apparatus. The parchment paper cap is replaced, and an additional pleated paper cap or hood, bearing the date of manufacture, is placed over the parchment paper.

After a close inspection to ascertain the presence of any foreign particles in the liquid, two wire ties are placed around the neck of the pleated paper cap and twisted tight.

All solutions are autoclaved the same day they are made. Bactriologic controls are placed in the autoclave at the same time.

A duplicate unit of collection bottle, mixing bottle, spherical flask, etc. is parallel to the first unit described. This permits two different solutions, or two batches of the same solution, to be made simultaneously. The distillate can be delivered to either receiving bottle by opening or closing a pinchcock.

*Made by American Sterilizer Co.

This equipment will produce large quantities of parenteral solutions per day. The iron framework was built by the hospital maintenance department at a comparatively small cost.

No paper filters are used.

The solution is protected from exposure to the air to a great degree.

CONCLUSION

An efficient as well as economical apparatus is described for preparing parenteral solutions.

AN ELECTRIC LANTERN SLIDE POINTER

NELSON A. MURRAY,* M.D., ROCHESTER, MINN.

THIS useful piece of apparatus can be made from odds and ends found in almost any laboratory and will well repay one for the few minutes spent in its construction. The pointer which I made was constructed from the battery case of a Mendelsohn flashlight gun, an adapter for the flashlight bulb, a cardboard mailing carton, two round wooden micro cover slip boxes, and a 7 diopter spectacle lens (Fig. 1). A 7 diopter lens is of approximately 14.28 cm. focal length. This focal length was chosen for no other reason than that it is a convenient length. A lens of 6 or 8 diopters will work equally well.

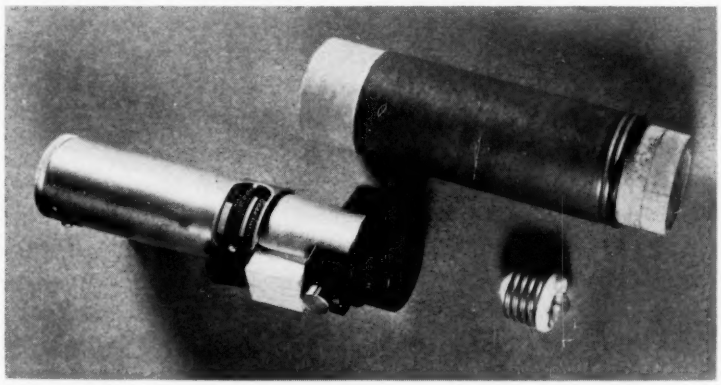


Fig. 1.—Material used in constructing the electric lantern slide pointer.

The lens is placed in front of the flashlight bulb so that the distance between the lens and the light source is slightly greater than the focal length of the lens (Fig. 2). The actual distance can be calculated readily from the standard lens formula. In order to set the lens for a spot at fifteen feet (500 cm.), calculations are as follows: $1/p + 1/q = 1/F$. When $p = 500$ cm. and $F = 14.28$

Received for publication July 27, 1942.

*Fellow in Pathology, Mayo Foundation.

cm., then q = 14.70 cm. Setting the lens at 14.70 cm. from the light source, a small, sharp spot will be focused at approximately fifteen feet. The pointer is adjustable so that by sliding the lens mount (coverslip box) in and out, the distance between the pointer and a sharp focused spot may be varied at will.

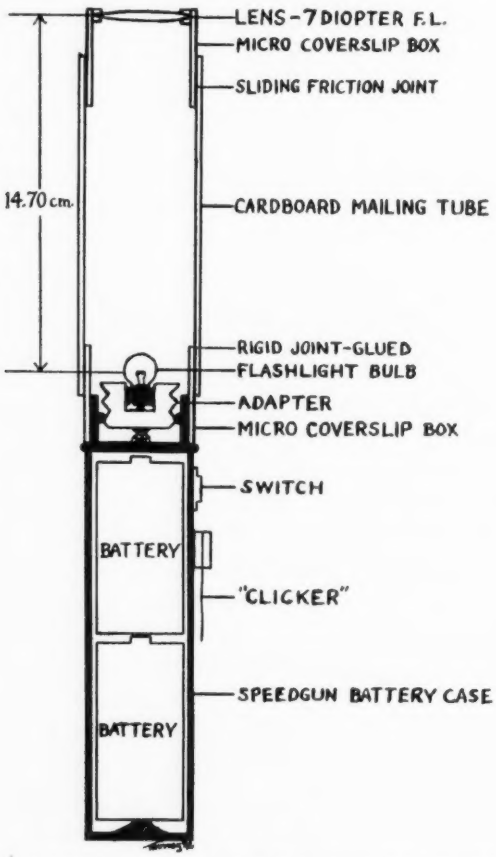


Fig. 2.—Diagram of the electric lantern slide pointer.

A piece of spring steel (slightly bent in the middle by tapping with a machinist's hammer) 1½ by ¾ by 1/32 inch (3.8 by 1.9 by 0.08 cm.) attached to the battery case, as illustrated, emits a "clicking" sound when pressed with the thumb and affords a convenient signal to the lantern operator to change to the next slide. Colored cellophane may be placed behind the lens if a colored spot is desired.

SS AGAR FOR THE ISOLATION OF EBERTHELLA, SALMONELLA, AND SHIGELLA GROUPS FROM FECES*

Compared With MacConkey and Bismuth Sulfite Agars and Tetrathionate Broth Followed by MacConkey Agar†

Mollie Mollov, M.S., Jeanette E. Winter, B.A., and Philip Steinberg, B.S.,
Jamaica, N. Y.

THE problem of routine isolation of Eberthella, Salmonella, and Shigella organisms from fecal specimens is one of vital importance from a public health standpoint. Detection of the etiologic agents is essential in the diagnosis of enteric bacillary disease and in the recognition of carriers. An efficient selective medium or combination of media is most important for the successful isolation of the incriminating organisms. Many types of media have been devised to facilitate the isolation of these organisms. Few, if any, have proved to be entirely satisfactory for all the enteric pathogens. The medium was often insufficiently inhibitive to prevent overgrowth of the coliform bacilli, or it failed to support the growth of some of the more fastidious organisms.

Liefson,[1] in 1935, devised the desoxycholate-citrate agar as a selective, inhibiting medium which enhanced the isolation of the Flexner type of *Shigella paradysenteriae* bacilli but which inhibited the Shiga, Sonne, dispar and alkalescens strains to a great extent. Paulson,[2] in 1937, found desoxycholate-citrate agar superior to the Endo and eosin-methylene blue agars in bowel disorders. Hardy and Watt[3, 4] confirmed the superiority of the desoxycholate-citrate medium in their experience with their Flexner dysentery cases. They mention, further, that the differential MacConkey agar compares favorably with the Endo and eosin-methylene blue but not with the desoxycholate-citrate agar in the isolation of Flexner dysentery bacilli. Cooper and associates[5] also conclude that MacConkey is superior to Endo and eosin-methylene blue but that desoxycholate-citrate is superior to all three for Flexner and also for Sonne dysentery isolations. Sellers, Morris, and Reynolds,[6] in 1934, found bismuth sulfite agar better than Endo for isolating *Eberthella typhosa*. Gunther and Tuft[7] concluded that bismuth sulfite is superior to Endo, eosin-methylene blue, and desoxycholate-citrate agar in the isolation of *E. typhosa* from urine and feces. Jones[8] found brilliant green-eosin agar superior to MacConkey for dysentery bacilli. Desoxycholate-citrate agar was found to be much better than MacConkey for isolating dysentery bacilli by Irons and co-workers[9] in 1939. Difco Laboratories, Inc.,[10] in 1939, revised their formula for MacConkey agar to support the growth of all the dysentery as well as the typhoid and Salmonella organisms.

*From the Bacteriology Department and the Department of Pathology, Queens General Hospital, Jamaica, Long Island, N. Y.

Received for publication, July 29, 1942.

†SS, bismuth sulfite, and MacConkey agars and tetrathionate broth used in this study were products purchased from the Difco Laboratories.

Studies with SS (Shigella-Salmonella) agar were first reported by Mayfield and Gober[11] 1940. They found SS agar to be as efficient as desoxycholate-citrate for the isolation of dysentery bacilli. They mention, further, that the pathogenic and nonpathogenic* colonies were more clearly defined on the SS medium. Rose and Kolodny[12] confirmed these findings in their studies of Flexner and Sonne dysentery cases. Cooper, Keller and Glesne,[13] in 1942, reported the superiority of SS and desoxycholate-citrate agars over the MacConkey agar in cases caused by *Shigella paradysenteriae* Flexner. They stated, further, that MacConkey was slightly more efficient in isolating Sonne dysentery organisms and also, that although the SS and desoxycholate-citrate agars were equally efficient in the isolation of Flexner dysentery bacilli, a simpler and more distinct differentiation on SS of proteus organisms were present, thus eliminating need for many subcultures.

Several types of *liquid media* have been described to enhance the growth and facilitate the isolation of pathogenic intestinal organisms. In 1936 Leifson[14] described the combination of selenite F enrichment and desoxycholate agar for the isolation of typhoid bacilli. The sodium acid selenite salt favors the growth of typhoid and paratyphoid bacilli and inhibits the coliform organisms. In 1923 Mueller[15] demonstrated that tetrathionate broth, also a selective fluid medium, inhibited the coliform bacilli and permitted the growth of typhoid and paratyphoid bacilli. Kaufman[16] in 1936 increased his positive findings of *E. typhosa* and members of the Salmonella group with this medium. In 1939 Difco Laboratories, Inc.,[17] recommended the use of their tetrathionate broth for enriching stool specimens for the isolation of typhoid, paratyphoid, and dysentery bacilli.

We wish to offer our observations on 501 fecal specimens cultured on SS and MacConkey agars and tetrathionate broth and to compare the findings of our positive cases with the media employed. We wish to report the efficiency of SS agar in isolating many members of the Shigella and Salmonella groups and many strains of *E. typhosa*.

We would also like to mention our results with SS, bismuth sulfite, and MacConkey agars in the isolation of *E. typhosa* from feces in a few known cases of typhoid and one known typhoid carrier.

MATERIAL USED

Fecal cultures of dietary workers, nurses, and interns were examined routinely to rule out carriers of intestinal pathogens. Fecal specimens of hospital cases showing bowel disorders, as diarrhea and the like, were also cultured. In no instance in this study had we any indication in the primary culture of the patient as to whether the specimen contained enteric pathogens. Where no pathogens were isolated from any of the media employed, the case was considered negative either because of the inadequacy of our media or the absence of organisms. The specimen that showed enteric pathogens on all or any of the media employed was considered positive. We wish to compare the results of these positive cases for the evaluation of the media used.

*In this paper, by pathogenic organisms we refer to those belonging to the Eberthella, Salmonella, and Shigella groups.

TECHNIQUE

Fresh fecal specimens were sent to the laboratory in clean paper cups fitted with tight covers. Inoculations were made onto one plate each of SS agar and MacConkey agar and into one tube of tetrathionate broth. Each medium was inoculated separately. A large inoculum was placed on the SS plate and streaked freely over the surface. A large inoculum was put into the 10 c.c. of tetrathionate broth to which was added, just before use, 0.2 c.c. of an iodine solution, which was made up of 5 Gm. of potassium iodide and 6 Gm. of iodine to 20 c.c. of distilled water.* A much smaller inoculum was streaked on the MacConkey agar, often diminishing the inoculum, after making two to three streaks over part of the surface by cutting through the agar with the platinum wire at the outermost edge of the medium and then continuing to streak over the unused surface of the plate. All three media were incubated at 37° C. The agar plates were read after eighteen to twenty-four hours, and the number and character of the nonlactose-fermenting colonies on each solid medium were noted and recorded. Two to four of each type of nonlactose fermenting colony were fished to Krumweide sugar slants from each plate. The tetrathionate broth was streaked to MacConkey agar after incubation. This plate was in turn incubated overnight and treated as the initial agar plates. One Krumweide triple sugar slant from each case, giving typhoid, paratyphoid, or dysentery reactions was studied in detail. The other Krumweide slants were kept at icebox temperature until the identification of the slant was completed. Subcultures were made to phenol red broth fermentation tubes (lactose, saccharose, dextrose, mannitol, maltose, sorbitol, dulcitol, salicin). Tryptone broth was inoculated for indol production. Motility tests were done on young cultures from nutrient broth. Motility test medium, a semisolid agar† was used when broth cultures failed to show motility. Fermentation tests were read at twenty-four and seventy-two hours and then intermittently for ten days. All Shigella organisms were identified serologically by us and checked by the New York City Health Department. The Salmonella organisms were identified serologically by the International Salmonella Committee at the Beth Israel Hospital, New York. All final identifications were based on cultural and serologic findings. The organism from one Krumweide slant from each specimen was studied in detail. However, if the first Krumweide triple sugar slant chosen gave atypical reactions, another slant, fished at the same time from another suspicious colony, was carried through the same procedure detailed above.

In our studies of the known typhoid cases and the known typhoid carrier, in addition to the SS and MacConkey media, a bismuth sulfite agar plate was used. A large inoculum was streaked freely over the entire surface of the medium, and the plate was incubated at 37° C. for forty-eight hours. This plate was then examined, two to four characteristic black colonies were fished to Krumweide triple sugar slants, and the same procedure as described above was followed.

RESULTS

Five hundred and one unknown fecal specimens were examined. Three hundred and thirty-three were from dietary workers, nurses, and interns ap-

*Recommended by the Difco Laboratories, Inc.
†Difco Laboratories, Inc.

parently in good health. One hundred and sixty-eight were from hospital patients with possible intestinal disorders. Of the 333 specimens mentioned above, fourteen specimens were positive. These were isolated from five individuals who were now labelled carriers of typhoid, Sonne, atypical dysentery one case each), and alkalascens (two cases each). For purposes of studying the efficiency of the culture media employed, we are listing each specimen as a separate case. Of the 168 hospital cases, twenty-six specimens were positive from sixteen cases. Table I lists the types of organisms in the Eberthella, Salmonella, and Shigella groups found to grow on SS agar and also compares the nonlactose fermenting colonies noted in each case, quantitatively, on each type of medium used in this study. It is significant to note that *of the forty positive cases listed, thirty-four were isolated on SS agar only, twenty-seven in tetrathionate broth followed by MacConkey agar, and seventeen on the MacConkey*

TABLE I

COMPARATIVE RESULTS WITH SS AND MACCONKEY AGARS QUALITATIVE RESULTS WITH TETRATHIONATE BROTH

CASE OR CARRIER	ORGANISMS	NUMBER OF PATHOGENIC COLONIES ON		TETRA
		SS	MAC	
1. Case (S. Z.)	E. typhosa	Many	None	Positive
2. Case (R. W.)	E. typhosa	Many	None	No growth
3. Carrier (G. C.)	E. typhosa	Overgrown	Overgrown	Positive
4. Carrier (G. C.)	E. typhosa	Many	Many	Positive
5. Carrier (G. C.)	E. typhosa	Numerous	Overgrown	Positive
6. Carrier (G. C.)	E. typhosa	Numerous	Overgrown	Positive
7. Carrier (G. C.)	E. typhosa	Many	Overgrown	Positive
8. Carrier (G. C.)	E. typhosa	Many	Few	Positive
9. Case (H. D.)	E. typhosa	Few	Numerous	No growth
10. Case (H. D.)	E. typhosa	Many	Overgrown	Negative
11. Case (H. D.)	E. typhosa	Many	Overgrown	Negative
12. Case (H. D.)	E. typhosa	Many	None	Positive
13. Case (L. G.)	E. typhosa	Few	Many	Positive
14. Case (L. G.)	E. typhosa	Numerous	Numerous	Positive
15. Case (L. G.)	E. typhosa	Many	Numerous	Positive
16. Case (L. G.)	E. typhosa	None	Few	Negative
17. Carrier (G. A.)	Sh. Sonne	Many	None	Negative
18. Carrier (D. M.)	Sh. Sonne	Few	Few	Positive
19. Carrier (D. M.)	Sh. Sonne	Occasional	None	Negative
20. Case (J. G.)	Sh. Flexner	Many	None	No growth
21. Case (J. G.)	Sh. Flexner	Few	None	No growth
22. Case (J. G.)	Sh. Flexner	Few	None	Negative
23. Case (W. M.)	Sh. Flexner	Many	None	Negative
24. Carrier (B. M.)	Sh. alkalescens	Many	Few	Negative
25. Carrier (B. M.)	Sh. alkalescens	Few	Few	Positive
26. Carrier (X. M.)	Sh. alkalescens	Many	Occasional	Positive
27. Carrier (X. M.)	Sh. alkalescens	Few	None	Positive
28. Carrier (G. R.)	Sh. atypical	None	None	Positive
29. Case (G. A.)	Sh. atypical	Many	None	Negative
30. Case (F. H.)	S. schottmülleri	Numerous	Few	Positive
31. Case (E. D.)	S. montevideo	None	None	Positive
32. Case (T. J.)	S. newport	Many	Few	Positive
33. Case (M. S.)	S. cholerae suis var. Kuzendorf	Many	Few	Positive
34. Case (W. T.)	S. typhi murium	Many	Few	Positive
35. Case (W. Z.)	S. oranienberg	Overgrown	Overgrown	Positive
36. Case (W. Z.)	S. oranienberg	Many	Overgrown	Positive
37. Case (W. Z.)	S. oranienberg	Occasional	Overgrown	Positive
38. Case (B. E.)	S. oranienberg	Many	Many	Positive
39. Case (G. C.)	S. urbana	Few	Occasional	Positive
40. Case (C. W.)	S. derby	None	None	Positive

agar. In eight cases, the SS agar only was positive, and in five instances, only the tetrathionate picked up the intestinal pathogens. In two of these five cases, the SS and MacConkey agars were overgrown by coli because too large an inoculum was used. In one case only was the MacConkey plate alone positive. It will be noted further that SS was as efficient or superior to MacConkey for all but one case of typhoid and for many types of Shigella and Salmonella. Table II illustrates the total number and percentage of positive cultures on the various media used, and Table III illustrates the number and percentage of positive findings with the various combinations of the three media.

TABLE II

NUMBER OF PERCENTAGE OF POSITIVE CULTURES WITH THE VARIOUS MEDIA

ORGANISMS	TOTAL NUMBER	NUMBER OF POSITIVE CULTURES ON		
		SS	MAC	TETRA
E. typhosa	16	14	7	11
Sh. Sonne	3	3	1	1
Sh. Flexner	4	4	0	0
Sh. alkalescens	4	4	3	3
Sh. atypical	2	1	0	1
S. schottmülleri	1	1	1	1
S. montevideo	1	0	0	1
S. newport	1	1	1	1
S. cholerae suis var Kuzendorf	1	1	1	1
S. typhi murium	1	1	1	1
S. oranienberg	4	3	1	4
S. urbana	1	1	1	1
S. derby	1	0	0	1
Total positive cultures	40	34	17	27
Per cent of positive cultures	100	85	44.4	67.4

TABLE III

COMPARISON OF TOTAL POSITIVE FINDINGS WITH EACH MEDIUM AND WITH VARIOUS COMBINATIONS OF MEDIA

	TOTAL POSITIVE	PER CENT POSITIVE
SS agar	34	85.0
SS plus MacConkey agar	35	87.5
SS plus tetrathionate broth	39	97.5
MacConkey agar	17	44.4
MacConkey agar plus tetrathionate broth	29	72.5
Tetrathionate broth	27	67.4
SS and MacConkey agar and Tetrathionate broth	40	100.0

It is of interest that SS agar aided in the diagnosis of 85 per cent of the positive cases, and the combined use of SS agar and tetrathionate broth allowed successful isolations in 97.5 per cent of the studied cases. In contrast, we find MacConkey agar successful in the isolation of 44.4 per cent of the cases, and the combined use of MacConkey agar and tetrathionate broth successful in 72.5 per cent of the cases studied. It is of significance that SS agar in conjunction with tetrathionate broth allowed us to diagnose all but one of these forty cases.

DISCUSSION

We have run the gamut of culture media for the detection of intestinal pathogens from feces in our laboratory. As a differential medium, MacConkey agar was found to be superior to eosin-methylene blue and Endo (the two most

commonly used types of media). This is in agreement with the published results of Hardy and Watt, Cooper and co-workers, and others. It is the most clear-cut differential medium so far described which supports the growth of all intestinal pathogens and coliform bacilli. The coli colonies are brick red surrounded by a zone of precipitated bile. The colonies of Eberthella, Salmonella, and Shigella are uncolored and transparent and appear as clearing areas in the precipitated bile, caused by the coliform organisms. This makes possible a clear-cut reading when the plate is not overcrowded.

As a selective inhibitive medium we have found the SS agar superior to desoxycholate-citrate (unpublished). This is also in agreement with Mayfield and Gober and others. The SS medium gives maximum inhibition of coli with minimum restriction of the intestinal pathogens. It was found to support the growth of strains of Flexner, Sonne, alkalescens, and an atypical dysentery organism, many strains of *E. typhosa,* and a long list of many types of Salmonella, such as *S. newport, S. montevideo, S. cholerae suis* var. Kuzendorf, S. typhi murium, S. oranienberg, *S. urbana, S. schottmülleri,* and *S. derby.* This is an obvious advantage. Desoxycholate-citrate agar is recommended for the isolation of Flexner and Schmidt's dysentery bacilli but which restricts the growth of Shiga, Sonne, and alkalescens types of paradysentery bacilli. Bismuth sulfite is recommended to be superior to desoxycholate-citrate, eosin-methylene blue, and Endo agars for the isolation of *E. typhosa.*

In a very small series of known cases and one carrier of typhoid we have compared bismuth sulfite, SS and MacConkey agars. In all but one case have we found SS as efficient or superior to bismuth sulfite agar in isolating *E. typhosa* from feces, and in that case the MacConkey agar was also positive. Further study with these three media will be reported in a subsequent paper.

In SS agar we therefore find an excellent selective and advantageous inhibitive medium for the isolation of Eberthella, Salmonella, and Shigella organisms. It readily differentiates these three groups and other nonlactose-fermenting organisms which form opaque, transparent, or translucent uncolored colonies from the brick red-colored coli. Of all the inhibitive, selective media thus far described, SS is one medium which is sufficiently inhibitive to allow a large inoculum and which supports the growth of all intestinal pathogens found in these studies.

The tetrathionate broth has been very useful. In five of the thirty-one positive findings, the organisms were picked up only in the tetrathionate broth. In three of these cases no pathogens were seen in either of the agars used. In two cases, both the SS and MacConkey plates were overgrown by coli. Where there exist too few organisms in the specimen to be recognized on agar, they may multiply in the tetrathionate broth, which inhibits the coli, and then on subculture, from this broth, the pathogens may be more easily isolated. It is also of value in cases where, unfortunately, too large an inoculum is used on solid media and, as a result, discrete colonies are not obtained. Tetrathionate broth was found to support the growth of many strains of Salmonella, Shigella, and Eberthella as listed in Table I.

CONCLUSION

1. SS agar is an excellent culture medium for isolating Eberthella, Salmonella, and Shigella from feces. It gives fine definition of colony, provides for maximum inhibition of coli, and facilitates isolation of intestinal pathogens when a large inoculum is used.

2. If one selective, inhibitive, solid medium were to be chosen for the isolation of all intestinal pathogens, SS agar would seem to be best choice thus far.

3. In conjunction with SS agar, tetrathionate broth is of definite value in the isolation of Eberthella, Salmonella, and a few of the Shigella group.

4. In a small series of positive cases, SS agar was found as efficient or superior to bismuth sulfite agar in the isolation of *E. typhosa* from feces.

We wish to thank Dr. Alfred Angrist, Pathologist of Queens General Hospital, for his many helpful suggestions and kind cooperation.

REFERENCES

1. Liefson, E.: New Culture Media Based on Sodium Desoxycholate for the Isolation of Intestinal Pathogens and for the Enumeration of the Coli Bacilli in Milk and Water, J. Path. & Bact. **40**: 581, 1935.
2. Paulson, Moses: The Clinical Use of Desoxycholate and Desoxycholate-Citrate New Culture Media for the Isolation of Intestinal Pathogens, Am. J. M. Sc. **193**: 1, 1937.
3. Hardy, A. V., and Watt, J.: Acute Diarrheal Disorders, Am. J. Pub. Health **28**: 730, 1938.
4. Hardy, A. V., Watt, T., DeCapito, M., and Kolodny Maxwell, H.: Studies of Acute Diarrheal Diseases. 1. Differential Culture Media, Pub. Health Rep. **54**: 287, 1939.
5. Cooper, Merlin L., Furcolow, M. L., Mitchell, A. Graeme, and Cullen, Glenn E.: The Relation of Dysentery to the Acute Diarrhea of Infants and Children, J. Pediat. **15**: 172, 1939.
6. Sellers, T. F., Morris, J. F., and Reynolds, M.: Notes on the Application of Bismuth Sulfite Medium to the Isolation of B. Typhosus From Feces, J. Lab. & Clin. Med. **20**: 202, 1934.
7. Gunther, C. B., and Tuft, L.: A Comparative Study of Media Employed in the Isolation of Typhoid Bacilli From Feces and Urine, J. Lab. & Clin. Med. **24**: 461, 1939.
8. Jones, E. R.: Use of Brilliant Green-Eosin Agar and Sodium Tetrathionate Broth for the Isolation of Organisms of the Typhoid Group, J. Path. & Bact. **42**: 455, 1936.
9. Irons, J. V., Bohls, S. W., DeShazo, Thelma, and Hewlett, L. L.: Observations on MacConkeys' and Desoxycholate-Citrate Agars for the Isolation of Dysentery Bacilli, J. Lab. & Clin. Med. **25**: 81, 1935.
10. Difco Manual: A New MacConkeys' Agar, 1939.
11. Mayfield, R. Catherine, and Gober, Maud: Comparative Efficiency of Plating Media for the Isolation of Shigella Dysenteriae, Am. J. Pub. Health **31**: 363, 1941.
12. Rose, Harry M., and Kolodny, Maxwell H.: Use of SS (Shigella-Salmonella) Agar for the Isolation of Flexner Dysentery Bacilli From the Feces, J. Lab. & Clin. Med. **27**: 1081, 1942.
13. Cooper, Merlin L., Keller, Helen M., and Glesne, Lillian R. B.: Bacillary Dysentery in Infants. Evaluation of Three Media, Sodium Desoxycholate-Citrate, SS and MacConkey for the Isolation of Shigella Paradysenteriae From Stools, J. Pediat. **20**: 596, 1942.
14. Liefson, E.: New Selenite Enrichment Media for the Isolation of Typhoid and Paratyphoid (Salmonella) Bacilli, Am. J. Hyg. **24**: 423, 1936.
15. Mueller, L.: Un Nouveau Milieu d'enrichissement pour la Recherche de Bacilli Typhique et des Paratyphiques, Compt. rend. Soc. de biol. **89**: 434, 1923.
16. Kaufmann, F.: Ein Kombiniertes Anreicherungsverfahren fur Typhus- und Paratyphus-Bazillen, Zentralbl. f. Bakt. I Abst. Orig. **119**: 148, 1930.
17. Difco Manual: Tetrathionate Broth, 1939.
18. Tabit, F.: A Modification of Wilson and Blair's Bismuth Medium for Both Typhoid and Paratyphoid Bacilli, J. Path. & Bact. **46**: 181, 1938.
19. Coleman, Marion B.: The Differentiation and Identification of Bacillary Incitants of Dysentery, Am. J. Pub. Health **30**: 39, 1940.

CHEMICAL

SOME SOURCES OF ERROR IN SULFANILAMIDE DETERMINATIONS*

A. H. S. Holbourn, M.A., D.Phil., and R. E. Pattle, B.A.
Oxford, England

THE METHODS commonly adopted for the determination of the concentration of sulfanilamide in blood are liable to errors arising from the neglect of certain variables which strongly influence the values obtained. Until these variables were tracked down, and the errors due to their neglect eliminated, inconsistent results were obtained.

A number of modifications of Marshall's[1] original method have been proposed. But, as will be shown later, these have little or no effect on the errors with which this paper is concerned. These errors arise, for the most part, during the diazotization of the sulfanilamide, and, as the modifications to the original method are mainly concerned with procedures subsequent to diazotization, they do not remove the errors which have already occurred.

As the particular method employed is therefore unimportant, a simple method, based on Marshall's original method, was used wherever possible. The exact procedure is described in the manual of the Evelyn photoelectric colorimeter as follows:

Take 0.2 c.c. whole blood, and add 7.8 c.c. distilled water. Allow to stand for two minutes. Add 2 c.c. 15 per cent toluenesulfonic acid. Mix well and filter. Pipette 4 c.c. of filtrate into a colorimeter tube, add 1 c.c. of 0.025 per cent sodium nitrite solution, mix well and stand for three minutes. Add 5 c.c. of a solution consisting of 1 c.c. dimethyl-α-naphthylamine in 250 c.c. ethyl alcohol. Read the color developed after ten minutes against a blank made up with 4 c.c. of 2.5 per cent toluenesulfonic acid instead of 4 c.c. of filtrate.

Determinations were also made using 10 per cent trichloracetic acid as a protein precipitant instead of toluenesulphonic acid, and 2 per cent trichloracetic acid in the blank instead of 2.5 per cent toluenesulfonic acid.

The sulfanilamide which was the subject of this investigation was added to the distilled water used for hemolyzing the blood in an amount equivalent to a concentration in the blood of 10 mg. per 100 c.c. Human blood, kept fluid by the oxalate crystal method, was used. A filter having a maximum transmission at 520 mμ was used in the colorimeter.

The most important of the numerical results on which the findings below are based are given in Table I. In the column headed "Density" is given 23.8

*From the Nuffield Department of Surgery, University of Oxford, England.
Received for publication, July 28, 1942.

TABLE I

TEMP. OF DIAZOT'N	DENSITY 3 MIN. DIAZOT'N		DENSITY 10 MIN. DIAZOT'N		DENSITY 20 MIN. DIAZOT'N		DENSITY 6 HR. DIAZOT'N	
	T.S.A.	TRICHL.	T.S.A.	TRICHL.	T.S.A.	TRICHL.	T.S.A.	TRICHL.
0° C.	3.5	4.3			9.2	9.7	9.7	
10°	5.7	7.2	9.3	10.0	10.3	10.5		
20°	9.0	9.7	9.7	10.0	10.0	10.0		7.0
40°	9.6	10.0			9.1	10.0		
20° DS	6.5	8.6			0.3	5.4		
20° PS20					10.0	10.0		
20° PS480					3.1	9.0		
20° U	7.4				9.0			

DS means that the diazotization was carried out in sunlight.

PS20 means that after the pink color had developed completely, it was exposed to 20 minutes of sunshine.

PS480 means that after the pink color had developed completely, it was exposed to 8 hours of sunshine.

U means that the nitrite was gently run in and not stirred.

$(2 - \log_{10}G)$, where G is the percentage transmission of the colorimeter tube. The effects of the following variables were studied.

The Effect of Light on the Diazo Reaction.—There was no perceptible difference between diazo reactions which proceeded in the dark and reactions which proceeded five feet away from a 60 watt bulb in a pearl glass shade, under any conditions of temperature or time of diazotization. But the effect of direct sunlight was very pronounced. The sun, in these experiments was always shining from a clear sky, but, unfortunately, this varied considerably in blueness, so that the different densities in the table are not strictly comparable. How important the effect of sunlight can be under certain conditions is shown by the fact that one solution which had been diazotized for twenty minutes in strong sunlight failed to produce any color at all when coupled to dimethyl-α-naphthylamine in the dark. Coupling to N-(1-naphthyl)ethylenediamine, instead of dimethyl-α-naphthylamine after twenty minutes' diazotization in sunlight caused a very faint yellowish color to appear instead of the usual purplish pink color. As one would expect, the effect of sunlight is less pronounced if one diazotizes in sunlight for only three minutes.

Once the diazo compound has been coupled to dimethyl-α-naphthylamine, and the pink color completely developed, twenty minutes' exposure to sunlight has no appreciable effect, although eight hours' exposure changes the pink color to a brownish yellow. According to Bratton and Marshall,[2] the purplish pink ethylenediamine compound is decomposed by sunlight.

Time and Temperature of Diazotization.—All the following results were obtained when the diazotization and the coupling were carried out in artificial light.

For temperatures of diazotization between 0° and 20° C., approximately the same maximum color was obtained on coupling if the diazotization had been carried out long enough. For temperatures between 10° and 20° C., ten minutes was sufficient to produce maximum color when trichloracetic acid was used as the precipitant, and twenty minutes when toluenesulphonic acid was used. A three-minute diazotization did not produce maximum color unless the temperature was raised to 30°. Too high a temperature or too long a time of diazotization produced some destruction of the diazo compound.

The Protein Precipitant.—It was found that with times and temperatures of diazotization which were insufficient to produce maximum color, trichloracetic acid produced somewhat denser color than toluenesulfonic acid. But when the time and temperature were sufficient to diazotize the sulfanilamide fully, the same color density was developed in the presence of either precipitant. With trichloracetic acid, diazotization was more rapid and it was less affected by sunlight, too high a temperature and too long a time of diazotization. Hence, from this point of view, trichloracetic acid is preferable as a precipitant.

The Keeping Qualities of Sulfanilamide in the Presence of Trichloracetic Acid or Toluenesulfonic Acid.—The sulfanilamide content remained constant when the protein free filtrate was kept in the dark at room temperature for fourteen days, with either precipitant.

The Keeping Qualities of the Alcoholic Solution of Dimethyl-α-Naphthylamine.—Perfectly consistent results could be obtained with a solution which had been kept at room temperature for a month. The solution became slightly brownish in this time, but, provided the blank was also made up with this brownish solution, the same readings for density were obtained as with a clear, fresh solution.

The Keeping Qualities of the Pink Dye.—Provided the diazotized sulfanilamide was coupled to the dimethyl-α-naphthylamine in moderate artificial light, full color developed in about ten minutes, for temperatures of coupling between 0° and 20° C. All readings were therefore taken after ten minutes. Even after keeping the pink dye in the dark at room temperature for a week the color was only just measurably darker than after ten minutes.

Improper Mixing of the Nitrite.—If the nitrite was not properly mixed with the protein free filtrate, diminished color was obtained even after a twenty-minute diazotization.

The Dilute Sodium Nitrite Solution.—The dilute nitrite solution (0.025 per cent) was always freshly made up each week from a 1 per cent stock solution. But it was afterwards found that some 0.025 per cent solution of ordinary sodium nitrite which had been kept in the dark for four months gave the same results as freshly made up solution, for concentrations of sulfanilamide equivalent to 10 mg. and also to 30 mg. per 100 c.c. Some dilute nitrite solution which had stood close up against a window for three months also gave the same results for 10 mg. per 100 c.c. No test was made with this nitrite solution on any stronger solution of sulfanilamide.

There was no difference in the results obtained when using A.R. sodium nitrite, and the results when using ordinary sodium nitrite.

Excess Nitrite and the Effect of Sunlight.—A very large excess of nitrite, say fifty times the usual excess, will inhibit the formation of the pink dye and lead to the formation of a yellowish color instead. Hence the excess nitrite is often removed with urea. When 1 c.c. of 0.25 per cent urea solution was added to a trichloracetic acid and sulfanilamide mixture which had been diazotized for twenty minutes in very strong sunlight, and the whole allowed to stand for one minute before coupling, practically no color was developed. Exactly the same low color density was obtained when the 1 c.c. of urea solution was replaced by 1 c.c. of distilled water. It was also found that when diazotization

had been carried out in the dark, the effects of 1 c.c. of urea solution and 1 c.c. of distilled water were exactly the same.

Use of N-(1-Naphthyl) Ethylenediamine.—Where a twenty-minute diazotization had taken place in very strong sunlight, a pale yellowish color developed on coupling to N-(1-naphthyl)-ethylenediamine instead of the usual purplish pink color. The removal of the excess nitrite did not change this color in any way. Similarly, when the diazotization was carried out in the dark, the removal of the excess nitrite had no effect.

DISCUSSION

Our results differ from those of previous workers in certain particulars of practical importance.

The most important result is the effect of sunlight on the diazo reaction. This does not seem to have been noticed before, possibly because diazotization for only three minutes is the usual practice. Unless the light was strong its effect in this amount of time might escape detection. Nevertheless the effect of light in tropical countries under camp conditions would be of the utmost importance whatever the time of diazotization.

Marshall[3] found that diazotization of sulfanilamide was practically complete in one minute, and no significant difference in color was obtained if the reaction was allowed to proceed for eight minutes. This does not agree with our results. A possible explanation is that Marshall's work may have been carried out at a room temperature considerably higher than usually obtains in Britain.

It is the general practice in diazotization to keep the temperature below 5° C., as most diazo compounds decompose rapidly above this temperature. The naphthylamines and nitroanilines, however, may be diazotized at room temperature without loss of yield. Sulfanilamide also appears to have a diazo compound which is comparatively thermostabile.

As, in our experiments, only free sulfanilamide was present, our results refer strictly only to free sulfanilamide determinations. Methods of determining total sulfanilamide are described by various workers. They are similar to the method given, except that the sample of blood or the protein free filtrate is boiled for about an hour either with the protein precipitant or with hydrochloric acid. This has little effect on subsequent operations, so that our results may be taken as applying without serious modification to determinations of total sulfanilamide.

We found that both trichloracetic acid and toluenesulfonic acid are satisfactory as protein precipitants. Marshall[3] found that both were satisfactory for free sulfanilamide, but that trichloracetic acid was unsatisfactory for total sulfanilamide because, owing to the decomposition taking place during boiling, it did not fully hydrolyze the conjugated compound. Hence Abrahamson[4] boils the filtrate with hydrochloric acid after precipitating the proteins with trichloracetic acid.

Marshall[1] recommends that the dilute nitrite solution should be freshly prepared, and that exceedingly pure nitrite should be used. We find these precautions unnecessary.

Marshall and Litchfield[5] propose certain improvements in the method, viz., removal of excess nitrite with ammonium sulfamate, and buffering of the reaction mixture with acid sodium phosphate. Ratish and Bullowa[6] use urea to remove the excess nitrite. We have found that the moderate excess of nitrite recommended in Marshall's original paper has no measurable effect in any circumstances in which we have made tests. It would certainly be surprising if, when the diazotization is incomplete due to too low a temperature or too short a time of diazotization, the removal of the excess nitrite or of any other of the suggested modifications of the original method completed the diazotization. Hence the time and temperature relationships given in Table I will remain the same.

In their latest method Bratton and Marshall[2] use N-(1-naphthyl)-ethylenediamine as coupling agent. Here they note the effect of light on the completed dye, but not on the diazo compound.

Marshall, Emerson and Cutting[7] find differences of 2-3 per cent between duplicate determinations. The figures given in the table have at least this degree of accuracy.

CONCLUSIONS

The generally recommended precautions have little or no effect in remedying the errors which often arise in the measurement of sulfanilamide. These errors are usually due to the fact that the diazo compound of sulfanilamide is very easily decomposed by light, and to the fact that, unless the room temperature is very high, diazotization is incomplete in three minutes, which is the usual time.

If the diazotization is incomplete, its dependence on time and temperature is very marked. Hence in order to avoid the necessity of accurate control of both the time and the temperature, the diazotization should proceed until it is as nearly complete as possible. Therefore, if trichloracetic acid is used as the protein precipitant, diazotization should proceed for ten minutes at temperatures between 0° and 30° C., but if toluenesulfonic acid is used, twenty minutes is necessary.

SUMMARY

When sulfanilamide is estimated by forming a diazo compound and coupling to dimethyl-α-naphthylamine or N-(1-naphthyl)ethylenediamine, it is recommended that the diazo reaction should proceed at room temperature in moderate artificial light for ten minutes in the presence of 10 per cent trichloracetic acid. With 15 per cent toluenesulfonic acid twenty minutes is necessary. The coupling reaction should also be performed in moderate artificial light.

The effect of sunlight might be very important when making determinations in tropical countries under camp conditions.

We should like to thank Mr. O'Brien and Mr. Higgins of the Radcliffe Infirmary for their valuable criticisms and assistance.

REFERENCES

1. Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, Proc. Soc. Exper. Biol. & Med. **36**: 422, 1937.
2. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.

3. Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, J. Biol. Chem. **122**: 263, 1937.
4. Abrahamson, E. M.: Office Clinical Chemistry, New York, 1940, Oxford University Press, p. 132.
5. Marshall, E. K., Jr., and Litchfield, J. T., Jr.: Determination of Sulfanilamide, Science **88**: 85, 1938.
6. Ratish, H. D., and Bullowa, J. G. M.: A Bedside Test for Sulfapyridine, J. LAB. & CLIN. MED. **25**: 654, 1940.
7. Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C.: Para-Aminobenzenesulfonamide; Absorption and Excretion: Method of Determination in Urine and Blood, J. A. M. A. **108**: 953, 1937.

A SIMPLE CHAMBER FOR ESTIMATION OF SERUM pH WITH A GLASS ELECTRODE*

ALFRED H. FREE, PH.D., CLEVELAND, OHIO

THE development of the glass electrode has led to its widespread use in the determination of pH or hydrogen ion concentration.[1] This electrode has been successfully employed in the measurement of the pH of blood, serum and plasma.[2-4] Recently many manufacturers of laboratory apparatus have placed on the market portable pH meters which are adapted for use with glass electrodes.

In the estimation of pH of blood, serum or plasma it is essential that the measurements be carried out in a closed chamber to prevent loss of CO_2 which will in turn cause changes in pH. Methods of handling blood to prevent CO_2 losses have been adequately discussed.[5, 6] The present report describes a simple chamber which can be used with a glass electrode in the determination of pH of blood serum.

Description of Chamber.—The chamber is made from a four-way glass stopcock in which the four outlets are in the same plane at right angles to one another. The plug of the stopcock has a bore such that any two adjacent outlets can be connected. The outlet tubes have an internal diameter of 1 mm. and 6 mm. external diameter and the plug of the stopcock has a 1 mm. bore. Such stopcocks are available from manufacturers.†

Onto one of the outlet tubes (Outlet II) is fused a larger tube which has a length of 30 mm. and an inner diameter of 13 mm. A larger tube (10 mm. inner diameter and 15 mm. long) is also fused onto the adjacent outlet (Outlet III) which is then bent at right angles as shown in the photograph (Fig. 1).‡

The chamber can conveniently be seated and fastened with rubber bands to a prong of wood which is attached to a small ring stand. The bulb type glass

*From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

Received for publication, September 15, 1942.

†Ace Glass Co., Vineland, N. J.; Corning Glass Works, Corning, N. Y.

‡A chamber with these specifications was made for the author by the Dietz Chemical Glass Works, Inc., 1975 E. 65th St., Cleveland, Ohio.

electrode is fitted into the upper chamber of Outlet II by means of a rubber collar placed around the neck of the glass electrode. This collar can conveniently be made from a rubber stopper or a piece of pressure tubing and should be air-tight. A piece of 20 gauge syringe needle is placed through the collar to serve as a gas exit. This exit can be readily closed by sticking a small cork on the needle as is shown in the photograph. The calomel half cell is connected to the side chamber by means of a rubber stopper. A small piece of rubber tubing is connected to the horizontal outlet tube (Outlet I). When the glass electrode and calomel cell are connected to the pH meter the instrument is ready for use.

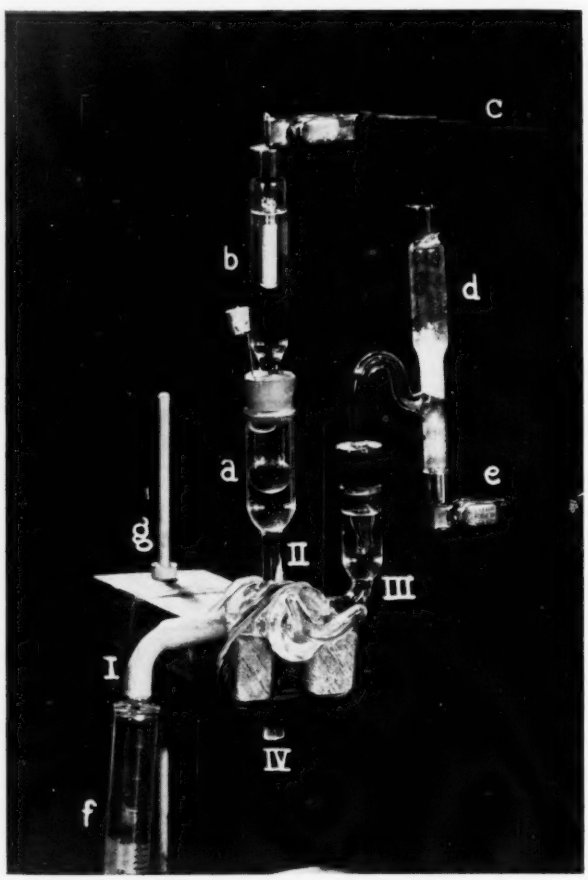


Fig. 1.—Chamber for determination of serum pH. *I*, Stopcock Outlet I for introducing serum sample. *II*, Stopcock Outlet II containing chamber into which bulb type glass electrode is inserted. *III*, Stopcock Outlet III containing chamber for attaching calomel half cell. *IV*, Stopcock Outlet IV which serves to carry away waste KCl and waste mineral oil. *a*, Chamber containing serum ready for measurement. *b*, Glass electrode. The bulb can be seen in the serum chamber. *c*, Lead to pH meter from glass electrode. *d*, Hellige calomel half cell. *e*, Lead from calomel half cell to pH meter. *f*, Syringe in position for introducing sample. *g*, Ring stand with small prolonged wooden block for holding chamber.

The chamber described has been used exclusively with a Hellige pH meter,* a Hellige calomel half cell, and a Hellige bulb type glass electrode (diameter of bulb 10 mm.). However, so far as can be ascertained, there is no reason why the apparatus could not be used with other instruments. It would no doubt be desirable to change the size and shape of the chamber housing the glass electrode if the glass electrode used were of a different design.

*Hellige electrometric pH meter with range control.

Method for pH Determination.—Using a 5 c.c. syringe which contains about 1 c.c. of mineral oil and no air bubbles, 2 c.c. of serum is drawn into the barrel of the syringe. The syringe is inverted so that the oil rises to the top and the needle is removed. The syringe is connected with the rubber tube on Outlet I and the stopcock turned to connect Outlets I and IV. With the syringe still inverted the oil is discharged through Outlet IV into a waste container. By carrying out this operation slowly the stopcock can be turned to connect Outlets I and II as soon as serum reaches the stopcock. The serum is forced into the chamber housing the glass electrode. The stopcock is then turned an eighth of a revolution to the right and a small cork placed over the end of the syringe needle which serves as a gas exit. The stopcock is then turned to connect Outlets II and III and the reading of the pH is made in this position. The stopcock is then turned to connect Outlets III and IV and approximately 2 c.c. of saturated KCL solution allowed to flow through the calomel half cell and out into a waste container. The serum is then allowed to flow out of the chamber by turning the stopcock to connect Outlets I and II and removing the stopper from the needle.

It has been found that if the serum drains completely there is no necessity to rinse out the serum chamber when a series of serum pH determinations are being made and the variation between samples is not more than 0.15 pH. It has furthermore been found that the loss of CO_2 from the serum when it is being introduced into the chamber is negligible. This has been found by introducing a sample and measuring its pH and subsequently withdrawing it through the needle exit while the second sample of the same serum is simultaneously being introduced. By this technique duplicates check to within 0.01 pH as they do when carried out in the conventional manner.

With care mineral oil can be kept out of the chamber and hence off the glass electrode. However, a small amount of oil deliberately introduced into the electrode chamber does not give a value different from a duplicate in which no oil is present.

For serum pH studies a convenient reference buffer is a $\frac{1}{15}$ M. phosphate buffer with pH of 7.38 at 38° C.[2] The buffer is standardized by exactly the same operations used for the serum.

In order to avoid temperature corrections the chamber and electrode system as well as the pH meter are kept at 38° C. This has been accomplished by keeping the whole outfit in a room at 38° C. or by placing the outfit in a large thermostat box maintained at 38° C.

SUMMARY

A simple chamber made from a four-way glass stopcock has been described for use with a glass electrode and pH meter in the determination of serum pH. The technique for measurement of serum pH with the chamber is described.

REFERENCES

1. Dole, M.: The Glass Electrode, New York, 1941, J. Wiley and Sons, Inc.
2. Havard, R. E., and Kerridge, P. T: An Immediate Acid Change in Shed Blood, Biochem. J. **23**: 600, 1929.

3. Stadie, W. C., O'Brien, H., and Laug, E. P.: Determination of the pH of Serum at 38° With the Glass Electrode and an Improved Electron Tube Potentiometer, J. Biol. Chem. **91**: 243, 1931.
4. Sendroy, J., Jr., Shedlovsky, T., and Belcher, D.: The Validity of Determinations of the pH of Whole Blood at Thirty-Eight Degrees With the Glass Electrode, J. Biol. Chem. **115**: 529, 1936.
5. Myers, V. C., and Muntwyler, E.: The Colorimetric Estimation of the Hydrogen Ion Concentration of Blood, J. Biol. Chem. **78**: 243-255, 1928.
6. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1931, Williams and Wilkins Co., Vol. II.
7. Hastings, A. B., and Sendroy, J., Jr.: Studies of Acidosis. XX. The Colorimetric Determination of Blood pH at Body Temperature Without Buffer Standards, J. Biol. Chem. **61**: 695, 1924.

NOTE ON THE DETERMINATION OF "CITRIN" IN LEMON PREPARATIONS*

ARTHUR E. GOLDFARB, M.D., ERNEST BUEDING, M.D., AND PHILIP KARP, B.S.
NEW YORK, N. Y.

THE expanding use of lemon extract containing so-called "Vitamin P" or "lemon citrin" in the treatment of psoriasis[1] makes it of interest to determine the "citrin" content of the lemon preparation used.

It was observed that in the determination of "citrin"[2] the reddish-brown color produced by addition of alkali to lemon preparations was increased by heating the samples for two minutes at 100° C. Since carbohydrates produce a brown color on heating with alkali (Moore's reaction), it was investigated whether the presence of sugar interferes with the determination of "citrin," and an attempt was made to eliminate this possible source of error.

The preparation of lemon extract was essentially the same as described by Lorenz and Arnold,[2] except that in one series of experiments the lemon peel was extracted without cooking.

Procedure.—In modification of the method described by Lorenz and Arnold,[2] the following procedure was used for the assay of "citrin" in lemon preparations:

To the solution to be analyzed, an equal volume of alcohol (95 per cent) is added and the mixture filtered. The filtrate is diluted with 50 per cent alcohol so that the color intensity lies within the optimum range of standard eriodictin solutions (lemon peel extract 1:100, lemon juice 1:20, final product being a mixture of both, 1:50). Eight-tenths c.c. of 25 per cent KOH is then added to 9.2 c.c. of this dilution and the color produced is read in the Klett-Summerson colorimeter using filter No. 42, at which time maximum color development occurs.

---

*From the Departments of Dermatology and Medicine, New York University, College of Medicine, the Dermatological Service of the Third (New York University) Medical Division, and the Medical Service of the Psychiatric Division, Bellevue Hospital, New York City.

Aided by a grant from The California Fruit Grower's Exchange, Inc., Mr. Anthony J. Lorenz—Director of Research.

Received for publication, Sept. 18, 1942.

Determinations of eriodictin solutions (A52) of known concentration showed that the optimum range falls between 0.04 and 0.2 mg. eriodictin per cubic centimeter in which a straight-line relationship is observed between concentration and colorimeter reading.

Effect of Glucose on Colorimetric Determination of "Citrin."—It was found that both lemon juice and lemon peel extract contain about 1 per cent of fermentable reducing monosaccharides. While this amount of glucose (0.05 per cent in a dilution of 1:20 of lemon juice) when added to standard eriodictin solutions does not produce any color reaction, a measurable color is produced with the above procedure after heating for two minutes. On the other hand, in all lemon preparations the apparent "citrin" value was higher after heating for two minutes than in the unheated sample. This indicates that on heating the samples, glucose and possibly other substances interfere with the "citrin" determination and, therefore, give higher values than correspond to the true "citrin" content of the lemon preparation.

Effect of Cooking on "Citrin" Content of Lemon Peel Extract.—The colorimetric determination of the cooked extract gives significantly lower values than the uncooked, indicating that some "citrin" is destroyed on cooking. The uncooked extract was obtained by allowing the ground peel to stand in cold water at room temperature for twenty-four hours or more.

"Citrin" Content of Lemon Juice.—A measurable quantity of "citrin" can be found in lemon juice; its concentration is about one-sixth of that contained in the uncooked peel extract. Whether the compound found in lemon juice has biological activity is yet to be determined.

When lemon peel extract and juice whose "citrin" contents have been determined separately are combined, the determination of this mixture yields the "citrin" value calculated from the figures for each component.

SUMMARY

1. A modification of the method of Lorenz and Arnold for the determination of "citrin" in lemon preparations is described, and the interference of glucose is eliminated by avoiding heating of the solutions after the addition of alkali.

2. A more efficient extraction of "citrin" from lemon peel is obtained by extracting in the cold instead of by cooking.

3. Lemon juice contains about one-sixth the amount of "citrin" found in lemon peel extract.

REFERENCES

1. Goldfarb, A. E.: Treatment of Psoriasis With Lemon Citrin (Vitamin P) With Lemonade and Ascorbic Acid, Arch. Dermat. & Syph. **43**: 356, 1941.
2. Lorenz, A. J., and Arnold, L. J.: Preparation and Estimation of Crude Citrin Solutions (Vitamin P) From Lemons, Food Research **6**: 151, 1941.

DETERMINATION OF URIC ACID IN WHOLE BLOOD AND SERUM*

J. W. Mull, Ph.D., Cleveland, Ohio

A STUDY of the uric acid content of the blood was undertaken with the threefold purpose of selecting a practical method for clinical determinations using a photoelectric colorimeter, establishing the normal range for uric acid by this method, and ascertaining the relation between the uric acid content of whole blood and of serum.

The colorimetric method of Benedict and Behre[1] on the silver chloride filtrate, while a most satisfactory method for a visual colorimeter, is unsuited to the photoelectric colorimeter since it must be read while hot. The method proposed by Brown[2] depends on exact timing, and with the great sensitivity of the photoelectric instrument the changes are so rapid as to preclude reading checks. That of Newton,[3] in addition to rather elaborate reagents, did not give a curve that could be duplicated with successive determinations on the standard. Although Folin's method,[4] permits the development of the color at room temperature, it requires too much time in the preparation of reagents to be available for clinical use. We therefore turned back to the simple, short, but direct method of Benedict,[5] to which we applied the uricase treatment of Blauch and Koch.[6] Since uricase destroys uric acid, the color developed after the addition of uricase represents impurities. This value subtracted from the untreated findings gives the true uric acid content.

METHOD

To separate 1 c.c. samples of whole blood and serum was added 25 mg. uricase powder. The tubes were then capped and allowed to incubate for two hours in a water bath held between 40 and 48 degrees C. Following incubation, the specimens, together with fresh 1 c.c. samples of the same whole blood and serum, were precipitated with 1 c.c. 10 per cent sodium tungstate, followed by 8 c.c. $\frac{1}{12}$ normal sulfuric acid. After thorough stirring, especially with the incubated tubes, they were allowed to stand ten minutes, then centrifuged.

A 2 c.c. aliquot of the clear supernatant fluid was pipetted into tubes and diluted to 10 c.c. with distilled water. To each was added 4 c.c. 5 per cent sodium cyanide, followed by 1 c.c. arseno-phosphotungstic acid. The tubes were inverted once and placed immediately in a boiling water bath for three minutes, removed from the bath and allowed to stand about one minute at room temperature, one minute in cold water, and were read after a total of three minutes and within the next five. A 650 mμ filter was used, reading on a logarithmic scale. Too rapid cooling or delay in reading usually resulted in clouding.

*From the laboratory of the Maternity Hospital and the Department of Biochemistry, Western Reserve University School of Medicine.

Received for publication, Sept. 21, 1942.

The 5 per cent sodium cyanide was made up in water containing 1 c.c. concentrated ammonium hydroxide per 500 c.c. The arseno-phosphotungstic acid was prepared by dissolving 100 Gm. sodium tungstate in 600 c.c. water, adding 50 Gm. pure arsenic pentoxide, 25 c.c. syrupy phosphoric acid (85 per cent H_3PO_4), and 20 c.c. hydrochloric acid in a liter flask. The mixture was boiled 20 minutes, cooled, and diluted to 1 L. This reagent keeps indefinitely.

The standard was prepared as recommended by Benedict and Hitchcock,[7] and contained 0.2 mg. per 1 c.c. This keeps at room temperature about one month if tightly stoppered and only opened infrequently. For use, 1 c.c. was diluted to 100 c.c. Samples of 1, 2, 4, and 5 c.c. of this diluted standard, corresponding to 1, 2, 4, and 5 mg. per 100 c.c. of blood, were used to establish a curve, plotting the electrophotometer readings against the concentrations. This curve was found to alter slightly with successive daily readings, and it was found necessary to repeat it every third day, using freshly diluted standard. The points on these curves invariably plotted in a straight line, the alteration being due to change in the 5 per cent sodium cyanide solution. Neither using different brand preparations of cyanide nor the addition of urea prevented this alteration, which was quite probably due to change in the ammonia concentration.

RESULTS

Employing the method as outlined, we examined fifty-one male medical students, twenty-two normal young women, and thirty-seven normal women in the last trimester of pregnancy. A complete summary of the findings on the male group is given in Table I. The means for the whole blood and serum uric

TABLE I

URIC ACID DETERMINATIONS ON 51 HEALTHY YOUNG MALES

(Values in Milligrams per 100 c.c. Blood or Serum)

	MEAN	S. D.	RANGE	EXCEPTIONS
Whole blood	3.39	0.77	2.05- 5.13	2 low, 1 high
Whole blood plus uricase	1.67	0.51	0.65- 2.67	0 low, 2 high
True uric acid	1.92	0.58	0.76- 3.02	1 low, 1 high
Serum	4.30	0.92	2.46- 6.14	2 low, 0 high
Serum plus uricase	1.07	0.42	0.23- 1.91	0 low, 1 high
True uric acid	3.23	0.60	2.03- 4.43	2 low, 0 high
Hemoglobin	15.51	1.23	13.05-17.97	2 low, 0 high

The range is determined by adding and subtracting 2 times the standard deviation from the mean; theoretically it includes 95 per cent of all determinations.

Correlation of the direct uric acid values of the whole blood and serum is plus 0.857.

Correlation of the true uric acid values of the whole blood and serum is plus 0.89.

Correlation of the direct whole blood uric acid values and the hemoglobin is minus 0.139.

acid, each of which is well over four times the standard deviation (S. D.), are significant, although the values are somewhat closer than had been anticipated. Moreover 95 per cent of the determinations made fall within the range according to statistical theory. There is some question, however, as to the significance of the means of the whole blood with uricase, the true uric acid of the whole blood, and the serum plus uricase, which are only about three times the standard deviation. The hemoglobin values are definitely significant, as the mean is well over twelve times the standard deviation. In the indirect method the correlation

figure of plus 0.89 between the amount of true uric acid in the whole blood and the amount in the serum indicates a definite relationship between the two. There is likewise a relation between the uric acid of the whole blood and of the serum in the direct method, with a correlation figure of plus 0.857. On the other hand, there is no relation between the uric acid of the whole blood and the hemoglobin, with a correlation value of minus 0.139.

TABLE II

COMBINED URIC ACID FINDINGS ON 51 MALES AND 22 FEMALES

(Values in Milligrams per 100 c.c. Blood or Serum)

	MEAN	S. D.	RANGE	EXCEPTIONS
Whole blood	3.53	0.707	2.11-4.94	2 low, 1 high
True uric acid	1.77	0.565	0.64-2.90	0 low, 4 high
Serum	4.14	0.866	2.41-5.87	2 low, 0 high
Serum plus uricase	1.19	0.393		
True uric acid	2.95	0.734	1.48-4.42	1 low, 1 high

Correlation of direct serum uric acid and the serum plus uricase values is plus 0.333.
Correlation of direct whole blood and true uric acid values is plus 0.727.
Correlation of direct serum and true serum uric acid values is plus 0.951.

A very brief summary of the combined findings of the young men and women are given in Table II. These means, from three to five times their respective standard deviations, are all significant. The ranges, while somewhat more restricted than those for the men alone, have no more exceptions than were found in Table I.

The most interesting observation is the correlation found between the uric acid as determined in the whole blood and the true uric acid, plus 0.951. This would indicate that, although there seems to be no relation between the direct determinations of uric acid and the impurities, as for example, the correlation value of plus 0.333 between serum and serum plus uricase, there is a definite relation between the direct determinations and the true uric acid. One is therefore justified in using the direct method on either whole blood or serum, provided only that the results are compared with the normal range established by this method.

TABLE III

DETERMINATIONS ON 37 WOMEN IN THE LAST TRIMESTER OF PREGNANCY

(Values in Milligrams per 100 c.c.—Volumes in Per Cent)

	MEAN	S. D.	RANGE	EXCEPTIONS
Uric acid in whole blood	3.21	0.648	1.90- 4.50	1 low, 3 high
True uric acid	1.19	0.504	0.18- 2.20	1 low, 1 high
Uric acid in serum	3.59	0.94	1.71- 5.47	0 low, 3 high
True uric acid	2.24	0.761	0.72- 3.76	0 low, 2 high
Hemoglobin	10.53	1.28	7.97-13.09	0 low, 0 high
Red cell volume	32.60	3.946	24.70-40.50	0 low, 2 high

Correlation of direct uric acid values in the whole blood and the red cell volume is minus 0.109.
Correlation of the direct uric acid values in the serum and the red cell volume is minus 0.161.
Correlation of the direct and true uric acid values of the whole blood is plus 0.89.
Correlation of the direct and true uric acid values of the serum is plus 0.97.

Table III summarizes the findings on pregnant women in the last trimester. Here again the whole blood and serum uric acid means are four to five times

their respective standard deviations, and therefore significant. While those of the true uric acid by the indirect method are of less definite significance, their range includes the theoretical proportion of the determinations. Both the hemoglobin and red cell volume means are definitely significant. There is no correlation between the uric acid either of the whole blood or of the serum with the red cell volumes. There is, however, excellent correlation between the whole blood and true uric acid, and between the serum and true uric acid, with values of plus 0.89 and plus 0.97 respectively.

In view of these findings, particularly that of the apparent correlation between the whole blood and serum uric acids, it would appear that uric acid determinations on whole blood are fully justified. The complete lack of correlation between whole blood uric acid and either hemoglobin or red cell volume, and also the absence of correlation between the serum uric acid and the hemoglobin or cell volume confirms the belief that it is unnecessary to use serum for uric acid.

TABLE IV

URIC ACID DETERMINATIONS ON INDIVIDUAL PREGNANCY CASES

(Values in Milligrams per 100 c.c. Blood or Serum)

	DATE	DIRECT WHOLE BLOOD	TRUE WHOLE BLOOD	DIRECT SERUM	TRUE SERUM	
D. M.	4/22/42	6.75	3.35	9.30	6.60	Near term
	4/23/42	5.40	2.40	7.85	5.45	
	5/ 6/42	3.30	1.40	5.45	3.95	
W. S.	9/ 2/42	9.60	7.3			Aborted at about 8 weeks
	9/ 8/42	3.20	2.2			

Table IV is included to illustrate further the correlation between the direct determinations of uric acid in whole blood or serum and the pure uric acid values found by the uricase method, even when the uric acid retention is above normal. The first case, D. M., was a diabetic near term; the second, W. S., had suffered a spontaneous abortion at about eight weeks, due to an Rh reaction, with a resulting retention that raised her blood urea nitrogen to 115 mg. per 100 c.c. In both cases the ratio of the values obtained with Benedict's direct method to the true uric acid values during retention is proportional, within the limits of correlation, to the ratio of the same determinations in the normal range. This further justifies the employment of the direct method.

SUMMARY

The direct method of Benedict was found most suitable for determining uric acid with the photoelectric colorimeter.

Either whole blood or serum may be used since there is a direct correlation between the findings.

There is also excellent correlation between the direct whole blood or serum determinations and the true uric acid findings made with the aid of uricase.

Ranges have been established for uric acid by both the Benedict direct method and the same method, after correction with the aid of uricase, for both

whole blood and serum, in males, males and females, and in normal pregnant women.

REFERENCES

1. Benedict, S. R., and Behre, J. A.: The Analysis of Whole Blood. III. Determination and Distribution of Uric Acid, J. Biol. Chem. **92**: 161, 1931.
2. Brown, H.: The Determination of Uric Acid in Blood, J. Biol. Chem. **68**: 123, 1926.
3. Newton, Eleanor B.: A Chromogenic Tungstate and Its Use in the Determination of the Uric Acid of Blood, J. Biol. Chem. **120**: 315, 1937.
4. Folin, O.: Standardized Methods for the Determination of Uric Acid in Unlaked Blood and Urine, J. Biol. Chem. **101**: 111, 1933.
5. Benedict, S. R.: The Determination of Uric Acid in Blood, J. Biol. Chem. **51**: 187, 1922. Benedict, S. R.: The Determination of Uric Acid, J. Biol. Chem. **54**: 233, 1922.
6. Blauch, Mary B., and Koch, F. C.: A New Method for the Determination of Uric Acid in Blood, With Uricase, J. Biol. Chem. **130**: 433, 1939.
7. Benedict, S. R., and Hitchcock, E. H.: On the Colorimetric Estimation of Uric Acid in Urine, J. Biol. Chem. **20**: 619, 1914-15.

QUANTITATIVE DETERMINATION OF BILE PIGMENT IN THE URINE*

A Comparative Study and Description of a Rapid, Direct, Photocolorimetric Method

Karl Singer, M.D., and Rosa Kubin, Ph.D., Boston, Mass.

DURING the last few years several methods for the quantitative determination of bile pigment in the urine have been published. Although such determinations may at present appear to be of but limited diagnostic value in the clinical study of liver diseases, they are of great importance for investigations of the hemoglobin and bile pigment metabolism in patients and experimental animals (renal biliary fistula dog[1]). Obviously, any quantitative determination of urinary bile pigment excretion should attempt to include all bile pigment compounds which could possibly be present. According to the view of Lemberg,[2] the primary bile pigment (not linked to globin) which develops in the course of hemoglobin catabolism, is not bilirubin but biliverdin (dehydrobilirubin). This substance, however, is immediately and completely reduced to bilirubin in the body, at least in man and dogs. In certain other species (frogs, birds) this reduction does not occur, and the color of the bile therefore is not brown but bluish green.[3] Under certain pathological conditions, e.g., in starvation (Thierfelder[4]), the reduction of biliverdin may also be incomplete and then the bile is composed of a mixture of both pigments. Furthermore, when bilirubin containing urine is exposed to light and air, a rapid, secondary reoxidation to biliverdin and sometimes to even higher oxidation products (choletelin) occurs. Thus, determinations of bilirubin only, are apt to give an inaccurate picture of bile pigment excretion.

At the present time the available quantitative methods for urinary bile pigments can be classified in two different ways. (1) according to the type of

*From the Department of Clinical Pathology, Middlesex University, Waltham, Mass.
Received for publication, Sept. 23, 1942.

chemical reaction used for the determination; (2) in regard to the preceding preparation of the urine for the performance of this chemical reaction, i. e., either separation of the bile pigments by precipitation, or direct determination in the urine without any preparation. As biliverdin cannot be diazotized, methods employing the van den Bergh reaction determine bilirubin only (Goodson and Sheard,[5] Scott,[6] Jendrassik and Czike,[7] and others[8]). If oxidation reagents are used, biliverdin besides bilirubin is included in the result (Hooper and Whipple,[9] Rous and McMaster,[10] and Knutti, Hawkins and Whipple[11 a, b]).

As the varying color of the natural urinary bile pigments (uroerythrin, urochrome, urobilin, etc.) interferes with the color obtained from the interaction between the bile pigments and the added reagents, most methods first separate the bile pigment compounds. Various precipitants are employed: calcium chloride (Hooper and Whipple[9]), barium salts (Scott[6]), trichloracetic acid (Kerpolla[12]). Such a precipitation is time consuming and increases considerably the inaccuracy inherent to any chemical performance. Recently Goodson and Sheard[5] found a way to avoid precipitation by excluding the disturbing natural pigments by means of an appropriate filter in the photoelectric colorimeter. This elegant method, therefore, permits a direct determination. Goodson and Sheard,[5] however, used the diazo reaction in their studies. Since we believe, for reasons which have already been broached, that oxidation methods are to be preferred in determining urinary bile pigments, we have worked out a direct photelometric method. This method is presented in this paper together with a comparative study of the other types of methods available for quantitative determination of urinary bile pigment compounds.

METHOD

Hammarsten's[13] reaction was used for the oxidation of the bile pigments. This reaction consists in the development of a persistent greenish-bluish color, when a mixture of hydrochloric and nitric acid in ethyl alcohol is added to fluids containing bilirubin or biliverdin. The reaction is quite analogous to the Gmelin test, but has the advantage that the oxidation remains arrested at a certain level and does not rapidly proceed further to colorless compounds, as is the case in the Gmelin test. This makes Hammarsten's[13] procedure suitable for colorimetric purposes.

Solutions Required.—(1) Hammarsten's reagent: the stock solution is prepared by mixing 19 parts of a 25 volume per cent hydrochloric acid with 1 part of a 25 volume per cent nitric acid. After twenty-four hours the stock solution is ready for use and then keeps indefinitely. For each test, 1 c.c. of the stock solution is diluted with 4 c.c. of absolute ethyl alcohol. (2) Blank solution: 7.5 c.c. of concentrated hydrochloric acid are diluted with water to make 1 liter.

Procedure.—The pH of the urine should first be tested with nitrazine paper. If the pH is higher than 6, a few drops of glacial acetic acid should be added to obtain an acid urine. Two test tubes or two graduated 10 c.c. cylinders are designated as "blank" and "test" respectively. Into each tube 1 to 2 c.c. of the centrifuged or filtered urine are then poured and 2 c.c. of 95 per cent alcohol are added, the contents are then well mixed by rotating the tubes. Then to the "blank" tube 2 c.c. of the blank reagent are added slowly, and to the "test" tube 2 c.c. of the diluted Hammarsten reagent. Both solutions, after

mixing again, are further diluted up to 10 c.c. by means of alcohol. The bluish-greenish color develops readily in the "test" tube and reaches its maximal intensity after two to five minutes. In the "blank," cloudiness caused by the precipitation of salts by the alcohol, may sometimes be noticeable. In this case the solution is filtered until completely clear. Occasionally it may be difficult to obtain a clear filtrate, in which case the "blank" may be prepared with distilled water instead of alcohol, without influencing the colorimetric evaluation.

Fisher's photoelectrocolorimeter (microset) was available in our laboratory. The red filter (650 mμ) was used. Although no special spectrophotometric studies could be performed, our results confirm the findings of Goodson and Sheard[5] that the disturbing influence of the various urinary pigments can be eliminated by the use of an appropriate filter if the diluted, untreated and acidified urine is employed as a standard for the photelometric comparison. The "zero-point" was determined with ethyl alcohol. Then the "blank" was read and the figure obtained was subtracted from the figure of the "test" solution.

Standardization.—From a stock solution of 20 mg. of bilirubin (Pfannenstiel) in 100 c.c. chloroform, convenient series dilutions were prepared and the oxidation tests performed. The photelometric readings were plotted in a calibration curve on semilogarithmic paper. Like the "diazoreaction" curve, the "oxidationreaction" curve obtained, is not a strictly straight line. The most reliable readings are obtained for a concentration of 0.15 to 0.4 mg. bile pigment per c.c. of urine. If the concentration in a urinary sample should be found out of this range, adaptation to the test by adequate increase or decrease of the amount of urine used is recommended.

COMPARATIVE STUDY

Urine samples from four dogs with renal biliary fistulas* and from four patients with extrahepatic obstructive jaundice were used for the comparison of the above described method with three other types of determination of biliary pigment. These other methods were (1) the direct diazo method of Goodson and Sheard,[5] in which caffeine sodium benzoate is employed as an accelerator in the development of the color reaction, (2) the method of Whipple and his associates,[11] employing calcium chloride for precipitation and nitric acid for oxidation, (3) Scott's[6] method using barium salts for precipitation and the diazo reaction for the determination. The results obtained with the four methods can be seen in Table I; each value therein is the arithmetic average of two parallel determinations.

In most of the experiments the highest values found were with the new direct oxidation method. In order to have a basis for a comparison of the different methods the results of the direct oxidation are assumed to equal 100 per cent and the other values are computed on this assumption (Table II).

DISCUSSION

1. *Comparison of the Two Direct Methods.*—(New oxidation method and diazo method of Goodson and Sheard,[5] Columns 3 and 4 of Tables I and II).

*We want to express our gratitude to Dr. Louis Bergmann and Dr. Leo Weisz for their cooperation in performing the operations.

Performance of the direct oxidation test requires only one-third of the time needed for the diazo test. Both methods give almost identical results with the urines of jaundiced patients (No. 11 to 14). In these experiments only freshly voided specimens were used. With fresh dog urine also, the same results were obtained (No. 8). In all the other animal experiments a twenty-four-hour urine was collected in a brown flask containing chloroform as a preservative,[11] the animal being in the metabolic cage. The diazo values, measuring bilirubin only, were always lower in these specimens, sometimes quite considerably. This is best explained as a result of the secondary reoxidation of the excreted bilirubin.

That bilirubin, when exposed to light and air, may rather rapidly disappear from a urinary specimen, has been repeatedly observed. (Rabinowitch,[14] Sivo and Forrai,[15] Scott[6]). Other factors, like temperature and ultraviolet rays, may also be of contributing importance (Peterman and Cooley[16]). The disappearance of bilirubin is caused mainly by reoxidation to compounds no longer giving the diazo reaction. The higher values of the oxidation method demonstrate clearly the superiority of this type of determination. However, the lower values with the diazo test may sometimes also be due to other causes. During the course of our experiments with the renal biliary fistula dogs almost daily determinations of the bile pigment output were performed using the method of Goodson and Sheard.[5] Immediately after operation the proper reddish-purplish color of diazobilirubin was obtained; later on, however, varying from a few days to a few weeks postoperatively, the diazoreagent, when added to the urine, produced only a brown or even a green color, unrelated to the pH of the specimen. Simultaneously with this phenomenon the urine began to contain great amounts of indican (highly positive Jolles and Obermeyer tests). That indican or related substances may interfere with the van den Bergh reaction in the plasma has been noted by previous investigators. Andrewes,[17] Harrison and Bromfield,[18] and also Becher[19] mention particularly the "buff or brown diazoreaction" in uremia where indican accumulates in the blood. The cause of the considerable indicanuria in our renal biliary fistula dogs was presumably the complete absence of bile from the intestine; bile was not administered to these animals because it would have interfered with a simultaneous investigation using these same dogs.

Two further questions deserve a brief discussion. (1) Does the Hammarsten reaction used in the direct oxidation test determine bile pigment compounds only? Hans Fischer and Adler[20] studied the different phases of the similar Gmelin test and found that other substances like malachite green, crystal violet, dimethylaminoparabenzol, etc., may give similar color compounds. All these substances are, however, unlikely to be present in any pathological urine. Indican, according to Naumann,[21] gives a very slowly developing bluish color with trichloracetic acid and Ferric chloride (Fouchet's reagent). We found in numerous urines not containing any bile pigment that indican, normal urinary pigments, and also stercobilinogen, prepared from feces, if added to the urine, did not give a positive Hammarsten test. Indican alone gives a brown color with the diazo reagent. (2) The second problem is whether the direct oxidation method determines all the excreted bile pigments or whether a loss occurs due to superoxidation to choletelin or colorless compounds. In order to answer this question dog

TABLE I

COMPARISON OF 4 METHODS FOR URINARY BILE PIGMENTS

COLUMN	1	2	BILIRUBIN IN MILLIGRAMS PER 100 C.C. OF URINE			
			3	4	5	6
			DIRECT METHODS		PRECIPITATION METHODS	
					CA CHLORIDE	BA ACETATE
	NO.	TYPE OF URINE	OXIDATION METHOD	DIAZO METHOD GOODSON-SHEARD	OXIDATION METHOD WHIPPLE AND ASSOC.	DIAZO METHOD SCOTT
	1	Dog 1	58.0	49.8	47.2	36.3
	2	Dog 1	59.5	54.2	42.6	39.8
	3	Dog 1	63.5	33.9	53.8	35.9
	4	Dog 1	45.0	31.2	47.0	27.5
	5	Dog 2	180.0	160.0	145.0	107.5
	6	Dog 2	155.0	137.5	140.0	131.3
	7	Dog 2	212.5	188.5	195.0	169.8
	8	Dog 3	66.9	67.3	not performed	
	9	Dog 3	125.0	80.0	100.5	73.3
	10	Dog 4	35.5	30.0	31.6	23.0
	11	Patient I	34.0	34.8	39.1	28.8
	12	Patient II	94.1	93.5	73.3	57.0
	13	Patient III	23.2	23.2	16.6	not performed
	14	Patient IV	17.4	17.4	not performed	

TABLE II

DIFFERENCES IN THE FOUR METHODS EXPRESSED IN PER CENT OF DIRECT OXIDATION METHOD

COLUMN	1	2	BILIRUBIN IN MILLIGRAMS PER 100 C.C. OF URINE					
			3	4		5		6
						PRECIPITATION METHODS		
						CA CHLORIDE		BA ACETATE
	NO.	TYPE OF URINE	DIRECT OXIDATION METHOD	DIRECT DIAZO METHOD GOODSON-SHEARD		OXIDATION METHOD WHIPPLE AND ASSOC.		DIAZO METHOD SCOTT
				%	DIFFERENCE	%	DIFFERENCE	%	DIFFERENCE
	1	Dog 1	100%	85.9	−14.1	81.4	−18.6	62.6	−37.4
	2	Dog 1	100%	91.1	− 8.9	71.6	−28.4	66.9	−33.1
	3	Dog 1	100%	53.3	−46.7	84.7	−15.3	56.5	−43.5
	4	Dog 1	100%	69.3	−30.7	104.4	+4.4	61.1	−38.9
	5	Dog 2	100%	88.9	−11.1	80.6	−19.4	59.7	−40.3
	6	Dog 2	100%	88.7	−11.3	90.3	− 9.7	84.7	−15.3
	7	Dog 2	100%	88.7	−11.3	91.8	− 8.2	79.9	−20.1
	8	Dog 3	100%	100.5	+0.5	not performed			
	9	Dog 3	100%	64.0	−36.0	80.4	−19.6	58.6	−41.4
	10	Dog 4	100%	84.5	−15.5	89.0	−11.0	64.8	−35.1
	11	Patient I	100%	102.4	+2.4	115.0	+15.0	84.7	−15.3
	12	Patient II	100%	99.4	−0.6	77.9	−22.1	60.6	−39.4
	13	Patient III	100%	100.0	0.0	71.6	−28.4	not performed	
	14	Patient IV	100%	100.0	0.0	not performed			

bile was taken from the gall bladder and the bile pigment content was immediately determined with both direct methods. Then the bile was protected against light and air by placing it in a dark bottle and adding mineral oil. In the fresh dog bile no biliverdin was present, as can be seen from the identical values with both the diazo and the oxidation tests (Table III). The values of the protected and also of the unprotected bile samples were then compared after twenty-four hours. Similar experiments were performed with urines. The results are compiled in Table IV.

TABLE III

EFFECT OF LIGHT AND AIR ON FLUIDS CONTAINING BILE PIGMENT

NO.	TYPE OF SAMPLE	IMMEDIATE DIRECT DETERMINATION		BILIRUBIN IN MILLIGRAMS PER 100 C.C. AFTER 20 TO 24 HOURS							
				PROTECTED FROM LIGHT				EXPOSED TO LIGHT			
		OXIDATION	DIAZO	OXIDATION		DIAZO		OXIDATION		DIAZO	
		MG. IN 100 C.C.	MG. IN 100 C.C.	MG. IN 100 C.C.	LOSS %	MG. IN 100 C.C.	LOSS %	MG. IN 100 C.C.	LOSS %	MG. IN 100 C.C.	LOSS %
1	Dog bile	1400	1408	1400	0	1236	12.2	1150	17.9	1134	19.5
2	Dog bile	860	880	860	0	360	59.1	495	42.5	430	51.1
3	Dog bile	1488	1488	1480	0.5	1280	13.9	1140	23.4	1060	28.6
4	Dog urine	68	58	67	1.5	56	3.4	67	1.5	51	12.1
5	Dog urine	157.5	137.5	157.5	0	120	12.7	131.3	16.6	100.5	27.0
6	Dog urine	212.5	188.2	177.5	16.5	147.5	21.6	157.5	25.9	122.5	34.9
7	Dog urine*	520	504	500	3.9	400	20.7	120	77	300	40.5
8	Dog urine*	330	335	320	3.0	234	30.2	216	34.6	148	55.8

*The high values occurred after injection of phenylhydrazine chloride.

TABLE IV

QUANTITATIVE RECOVERY OF BILIRUBIN ADDED TO URINE

SAMPLE NO.	MILLIGRAMS OF BILIRUBIN PER C.C.				
	IN URINE	IN BILIRUBIN SOLUTION	IN URINE PLUS BILIRUBIN		PERCENTAGE RECOVERED
			FOUND	ANTICIPATED	
1	none	0.20	0.190	0.200	95.0
2	none	0.26	0.250	0.260	96.1
3	none	0.37	0.380	0.370	102.7
4	none	0.55	0.560	0.550	101.8
5	none	0.89	0.810	0.890	91.0
6	0.135	0.17	0.273	0.305	89.5
7	0.140	0.17	0.279	0.310	90.0
8	0.320	0.17	0.510	0.490	104.1
9	0.137	0.30	0.430	0.437	98.4
10	0.770	0.21	0.950	0.980	96.9

Average recovery 96.6 per cent; range 89.5 to 104.1 per cent.

With one exception (No. 6, Table III) the value obtained with the oxidation test remained constant in the protected sample whereas all the other values decreased considerably. From these results it may be concluded that the oxidation test in samples adequately protected against superoxidation gives probably the most reliable picture of actual bile pigment excretion, although the possibility of a loss caused by superoxidation must always be kept in mind when balance studies of the pigment metabolism are performed.

2. *Comparison of the Two Oxidation Methods.*—(New direct method and precipitation method of Whipple and associates,[11] Columns 3 and 5, Tables I and II). The values estimated with the precipitation method showed deviations from those with the direct test covering a range from –28.4 per cent to +15.0 per cent, with an average deviation of –13.4 per cent. The standard deviation σ is 18.5 per cent. Most of the values with the precipitation method are lower than those with the simpler direct test. This is partly due to the loss to be expected from the more complicated chemical procedure, and perhaps partly caused by the difficulties in achieving the correct degree of oxidation of the bile pigments. The method of Whipple and his associates[11] requires an experienced worker as the amount of their oxidation reagent added to the urine

must be carefully adapted to the constantly varying concentration of pigment present, which factor may result in an insufficient or too efficient oxidation of the bile pigment compounds. These dangers are eliminated in the direct photelometric test. Peterman and Cooley[16] studied the various conditions of significance in obtaining maximal oxidation of bile pigments and recommended hydrogen peroxide as the most suitable oxidation reagent. Malloy and Evelyn,[22] following their suggestion, described a photo-electric method using hydrogen peroxide for determination of bile pigment in bile, feces and meconium. In our experience oxidation with hydrogen peroxide does not give satisfactory results with urinary bile pigment. Peterman and Cooley,[16] and Malloy and Evelyn[22] also expressed the opinion that the green color of fluids containing various bile pigments is not due to the presence of biliverdin. According to these authors biliverdin does not exist as a chemical entity but represents only a mixture of unoxidized yellow bilirubin and blue bilicyanin. This is in sharp contradiction to Lemberg,[2, 3] who defines biliverdin as a bile pigment compound having two hydrogen atoms less than bilirubin and an open α but an intact γ methene bridge. Both statements are most likely only at variance as far as the terminology is concerned. Biliverdin is a definite chemical substance but the green color of fluids containing bile pigment may also sometimes be caused by the presence of a mixture of unoxidized and oxidized pigments. In order to demonstrate that the direct oxidation test which yields a bluish-greenish color, does not leave any bilirubin undetermined, quantitative recovery experiments of bilirubin added to urinary samples were performed. The bilirubin was dissolved in chloroform and then added to urines with and without natural bile pigment. When performing the test, no technical difficulties were encountered, as chloroform in small amounts is readily soluble in alcohol, which is the main fluid medium of the test.

The recovery of bilirubin is as complete as can be expected from such an experiment. The range of the results was 89.5 per cent to 104.1 per cent and the average recovery 96.6 per cent. These values are almost identical with those found by Goodson and Sheard[5] in similar recovery experiments with their direct diazo method.

3. *Comparison of the Two Diazo Methods.*—(Direct method of Goodson and Sheard[5] and precipitation method of Scott,[6] Columns 4 and 6 of Tables I and II). If bilirubin only is to be determined in the urine, the direct oxidation method is doubtless the method of choice. The direct method is not only simpler to perform, but combines, as can be seen from the tables, this advantage with a much higher degree of accuracy.

CONCLUSIONS AND SUMMARY

1. Quantitative determination of urinary bile pigment excretion should attempt to comprise all bile pigment compounds which could possibly be present. Methods using the diazo reaction determine bilirubin only. Therefore oxidation methods which include also bile pigments other than bilirubin are to be preferred.

2. A direct, rapid, quantitative, photo-electric method, using Hammarsten's oxidation reaction, is described. By means of an appropriate filter and by

employing the diluted acidified but untreated urine as a standard, the otherwise necessary preliminary precipitation of the bile pigments can be eliminated.

3. A comparative study of this method with three other types of quantitative determination of urinary bile pigments is reported. The methods used in this comparison were (1) the direct diazo method of Goodson and Sheard, (2) the precipitation and oxidation method of Whipple and associates, (3) the precipitation and diazo method of Scott. Urines of renal biliary fistula dogs as well as of jaundiced patients were used in this study.

4. By means of the oxidation test and by an adequate protection against superoxidation, a reliable quantitative determination of the bile pigments excreted in the urine in twenty-four hours can be obtained, although the actually excreted amounts of bilirubin may decrease considerably in the collection period.

REFERENCES

1. Kapsinow, R., Engle, L. P., and Harvey, S. C.: Intra Abdominal Biliary Exclusion From the Intestines, Surg. Gynec. & Obst. **39**: 62, 1924.
2. Lemberg, R.: Animal Pigments, Ann. Rev. Biochem. **7**: 421, 1938.
3. Lemberg, R., and Wyndham, R. A.: Reduction of Biliverdin to Bilirubin in Tissues, Biochem. J. **30**: 1147, 1936.
4. Thierfelder, H.: Handbuch der physiologisch- und pathologisch-chemischen Analyse, Berlin, 1924, Julius Springer.
5. Goodson, W. H., and Sheard, C.: The Photelometric Determination of Bilirubin in Urine With Diazobenzene Sulfonic Acid, J. LAB. & CLIN. MED. **26**: 423, 1940.
6. Scott, L. C.: New Diazo Methods for Determination of Bilirubin in Blood and Urine, Brit. J. Exper. Path. **22**: 17, 1941.
7. Jendrassik, L., and Czike, A.: Bestimmung des Bilirubins im Blute, Ztschr. f. d. ges. exper. Med. **60**: 554, 1928.
8. Watson, C. J., Pass, I. J., and Schwartz, S.: A Study of the Supposed Conversion of Protoporphyrin to Coproporphyrin by the Liver, J. Biol. Chem. **139**: 583, 1941.
9. Hooper, C. W., and Whipple, G. H.: Bile Pigment Metabolism, Am. J. Physiol. **40**: 332, 1916.
10. Rous, P., and McMaster, P. D.: The Concentrating Activity of the Gallbladder, J. Exper. Med. **34**: 47, 1921.
11. a. Knutti, R. E., Hawkins, W. B., and Whipple, G. H.: Hemoglobin and Bile Pigment Overproduction in the Splenectomized Bile Fistula Dog, J. Exper. Med. **61**: 127, 1935.
    b. Sribhishaj, K., Hawkins, W. B., and Whipple, G. H.: Bile Pigment and Hemoglobin Interrelation in Normal Dogs, Am. J. Physiol. **96**: 449, 1931.
12. Kerpolla, W.: Ein kontrolliertes Verfahren zur quantitativen Bestimmung des Bilirubins, Acta med. Scandinav. **89**: 387, 1936.
13. Hammarsten, O.: Lehrbuch der physiologischen Chemie, Munich, 1922, J. F. Bergmann.
14. Rabinowitch, I. M.: Renal Threshold of Bilirubin, J. Biol. Chem. **97**: 163, 1932.
15. Sivo, R., and Forrai, E.: Ueber die Zersetzlichkeit von Bilirubinloesungen, Biochem. Ztschr. **189**: 168, 1927.
16. Peterman, E. A., and Cooley, T. B.: Studies on the Quantitative Estimation of Bile Pigments, J. LAB. & CLIN. MED. **19**: 723, 743, 1933.
17. Andrewes, C. H.: An Unexplained Diazo Color Reaction in Uremic Sera, Lancet **206**: 590, 1924.
18. Harrison, G. A., and Bromfield, R. J.: The Cause of Andrewes' Diazotest for Renal Insufficiency, Biochem. J. **22**: 43, 1928.
19. Becher, E.: Die Diazo—und Urochromogenreaktion im Blutfiltrat bei Niereninsuffizienz, Deutsches Arch. f. klin. Med. **148**: 10, 1925.
20. Fischer, H., and Adler, E.: Ueber Mesobilirubin und das Wesen der Gmelinschen Reaktion, Hoppe Seyler's Ztschr. f. physiol. Chem. **206**: 187, 1932.
21. Naumann, H. N.: A New Test for Bilirubin in the Urine, Biochem. J. **30**: 762, 1936.
22. Malloy, H. T., and Evelyn, K. A.: Oxidation Method for Bilirubin Determinations in Bile and Meconium With the Photoelectric Colorimeter, J. Biol. Chem. **122**: 597, 1938.

BOOK NOTICES

Hemorrhagic Diseases*

NYGAARD'S *Hemorrhagic Diseases* is a volume on blood coagulation and its mechanism, with a special discussion on the photoelectric method of determination of blood fibrin, also of the several hemorrhagic diseases. There is a discussion of vitamin K, but not of heparin.

Synopsis of Materia Medica, Toxicology and Pharmacology†

THE appearance of a second edition two years after the first speaks well for the popularity of Davison's *Synopsis of Materia Medica, Toxicology and Pharmacology.* It is rather difficult to visualize a 700 page book being a synopsis, but when compared with larger texts on the subject it emerges as truly synoptic in character. This brings us to the realization of the tremendous increase in our knowledge of the action of drugs and in the number of new drugs, in the past several years.

The volume is primarily clinical and serves as a ready reference manual.

Pathology of Trauma‡

THE pathology of trauma is a subject about which little is known to the average practitioner and medical student. Yet trauma ranks high in the list of causes of mortality and morbidity. In his book Dr. Moritz not only describes the pathology of trauma but also explains most lucidly why specific injuries take the form which they do. He calls upon an engineer's knowledge of forces, stresses, and vectors to make this clear to the reader. The book is not written from the point of view of the traumatic surgeon but from that of the pathologist and medical examiner. Consequently, it will be most valuable to persons in those fields. However, the background of knowledge which Dr. Moritz has drawn upon for the presentation of his subject is so broad that his book has a natural appeal to all physicians and students. The greater part of the book is devoted to a consideration of mechanical injuries to the various systems of the body, and emphasis is placed on the question of the predisposition of trauma to disease. This is not only a clinical problem but a most important medico-legal one, and in each instance Dr. Moritz reviews completely the evidence for and against trauma as an etiologic factor in disease.

Intelligent treatment of wounds demands a thorough knowledge of not only the anatomy but the pathology involved. The great demand today for young doctors who are trained to practice traumatic surgery on the battlefields makes it particularly fortunate that Dr. Moritz' book has appeared at this time. It is profusely illustrated, and will be a valuable teaching adjunct for those doctors preparing themselves for military duty.

---

*Hemorrhagic Diseases. Photo-Electric Study of Blood Coagulability. By Kaare K. Nygaard, M.D., Former Fellow in Surgery, the Mayo Foundation; Former Assistant Surgeon, the University Clinic, Oslo; Fellow of the Alexander Malthe Foundation for Research in Medicine, Surgery and Gynecology. Illustrated. Cloth, 320 pages. The C. V. Mosby Company, St. Louis, 1941.

†Synopsis of Materia Medica, Toxicology, and Pharmacology. For Students and Practitioners of Medicine. By Forrest Ramon Davison, B.A., M.Sc., Ph.D., M.B., Medical Department, The Upjohn Co., Kalamazoo, Mich. Formerly Assistant Professor of Pharmacology in the School of Medicine, University of Arkansas, Little Rock. Second Edition with 45 illustrations including 4 in color, 695 pages. The C. V. Mosby Company, St. Louis, 1942.

‡Pathology of Trauma. By Alan Richards Moritz, M.D. Cloth, 386 pages, with 117 illustrations, $6.00. Lea and Febiger, Philadelphia, 1942.

A Handbook of Allergy*

BLANTON'S *Handbook of Allergy* should fulfill well the purpose for which it is primarily intended. It is a synopsis type work for use by the undergraduate student, to be supplemented by personal instruction. It should also serve as a handy orientation volume for graduates who wish to become acquainted with the general subject of allergy. The discussion of treatment, both specific and symptomatic, is too abbreviated for the volume to serve adequately as a therapeutic reference aid. It is hoped that in the next edition treatment will receive more detailed discussion. When used as an undergraduate text, this becomes less of a problem since the student gains his therapeutic experience in the ward and clinic.

Directory of Medical Specialists†

THE second edition of the *Directory of Medical Specialists Certified by American Boards* is much larger than the first. A change in the page headings makes it a much more convenient reference volume. As previously, the specialties are arranged in alphabetical order. In the first edition page headings designated only the specialties. In the second edition the specialty appears on the left-hand page with the States on the right-hand and the cities scattered through the pages. This is a valuable reference volume.

Standard Nomenclature of Disease and Standard Nomenclature of Operations‡

THE *Standard Nomenclature of Disease* is now in its third edition. Many editions have been made, based primarily on the National Conference of Medical Nomenclature held under the auspices of The American Medical Association in 1940. For the first time there is also included a standard nomenclature of operations. Both sections are well indexed. Key numbers for all diseases and operations are arranged according to the punch card system so that with proper equipment any group or groups of diseases or operations may be sorted out with little expenditure of time.

The volume, published by The American Medical Association, will be especially valuable for teaching and research institutions, clinics, libraries, and statisticians.

Electrophoresis of Proteins§

ORIGINALLY a highly complicated phase of physical chemistry, electrophoresis has now entered much broader fields as an implement of investigation and has even found uses in clinical medicine. Most of us know it only by name but many are coming to realize that a more intimate acquaintance will be needed, as electrophoretic methods come to be applied in our own fields of study.

These authors describe general principles and methods of application and follow with discussion of studies of serum and plasma proteins, antibodies, antigens, enzymes, hormones, and the surface study of cells. The authors themselves have contributed a very great deal to this knowledge. The volume is necessarily still highly technical but recommended as the best reference volume on the subject that is available.

---

*A Handbook of Allergy for Students and Practitioners. By Wyndham B. Blanton, M.A., M.D., Litt. D., Professor of Clinical Medicine and Chief of the Immunology Clinic, O.P.D., Medical College of Virginia, Richmond, Virginia. Cloth, 191 pages. Charles C Thomas, Springfield, Ill., 1942.

†Directory of Medical Specialists. Certified by American Boards. 1942. Cloth, 2495 pages, $7.00. Published for the Advisory Board for Medical Specialties by Columbia University Press, New York, 1942.

‡Standard Nomenclature of Disease and Standard Nomenclature of Operations. Edited by Edwin P. Jordan, M.D. Cloth, 1022 pages. The American Medical Association, Chicago, 1942.

§Electrophoresis of Proteins and the Chemistry of Cell Surfaces. By Harold A. Abramson, Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University; Associate in Medicine, The Mount Sinai Hospital, New York City. Laurence S. Moyer, Assistant Professor of Botany, University of Minnesota, Minneapolis, and Manuel H. Gorin, Chemical Research Supervisor, Field Research Department, Magnolia Petroleum Company, Dallas, Texas. Cloth, 341 pages, $6.00. Reinhold Publishing Corporation, New York, 1942.

Outline of Histology*

HOSKINS and Bevelander's *Outline of Histology* is a loose-leaf laboratory book printed by the new photolithograph process. There are two columns to a page, facilitating easy reading, and many splendid illustrations. There is also an abundance of blank pages for notes and drawings. The volume should find wide use in the histological laboratory.

Drug Products†

HERRICK'S volume on *Drug Products* is a four hundred odd page description and analysis of the Federal Food and Cosmetic Act, designed to assist the manufacturer, distributor, and packer of drug products. It provides adequate interpretation of this complicated statute.

Human Pathology‡

THE sixth edition of Karsner's *Pathology* scarcely needs an introduction. However, there have been many changes from previous editions, enough to require resetting of type. The author and publishers have thereby taken advantage of the opportunity to make two column pages which facilitates reading. It has been abundantly and beautifully illustrated with 460 illustrations in black and white and 24 in color.

To Our Readers

The exigencies of the war situation have made it necessary to comply with the order of the War Production Board to reduce the weight of paper previously used for the JOURNAL. While this change will not affect the printed page it will affect the quality of the halftone illustrations. We regret that we have no choice in the matter and would ask the forbearance of our readers. As soon as the restrictions are removed, we shall resume our practice of printing the JOURNAL on the previously employed heavier grade of paper.

THE C. V. MOSBY CO., Publishers.

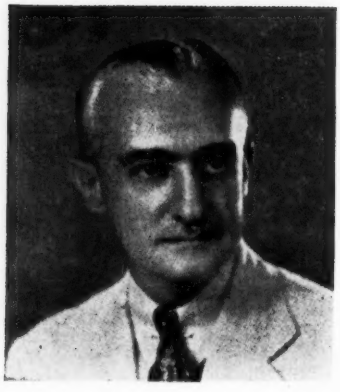
---

*Outline of Histology. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., Departments of Anatomy, College of Dentistry, and The Graduate School of Arts and Science, New York University. Paper, Pt. I., 179 pages, Pt. II, 113 pages, $2.50. The C. V. Mosby Company, St. Louis, 1942.

†Drug Products, Labeling Packaging Regulation. By Arthur Donald Herrick, Member of the New York and Federal Bar. Cloth, 466 pages, $7.50. Revere Publishing Company, New York, 1942.

‡Human Pathology. By Howard T. Karsner, M.D. Professor of Pathology, Western Reserve University, Cleveland, Ohio. 460 Illustrations in black and white and 24 subjects in color on 16 plates. Sixth Edition. Cloth, 817 pages, $10.00. J. B. Lippincott Company, Philadelphia, Montreal, London, 1942.

Simpson Mem. Inst



In Memoriam

Major Robert Anthony Kilduffe, M.C.

1889-1943

In charge of Laboratories, Ashford General Hospital
White Sulphur Springs, West Virginia
Associate Editor, The Journal of
Laboratory and Clinical Medicine, since 1924
Formerly
President, The American Society of Clinical Pathologists
Editor, The American Journal of Clinical Pathology
Director of Laboratories, The Atlantic City Hospital